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SECTION MEETINGS

CLEVELAND	
Western Reserve University	December 9, 1938
NEW YORK	
New York University Medical School	January 25, 1939
PACIFIC COAST	
University of California Medical School	December 7, 1938
SOUTHERN	
Tulane University	December 16, 1938
WESTERN NEW YORK	
Clifton Springs Sanitarium	December 10, 1938

10284 P

Reactions of the Electroencephalogram to Metrazol Injections.*

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In a paper published earlier by one of us[1] the epileptic attack induced by metrazol was studied by means of high speed photography and the cinematographic findings correlated with electromyographic records. This paper deals with the coördination of electroencephalographic (EEG) records taken in conjunction with the metrazol seizure, and with our previously published findings.

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* This project was aided by a grant from the Child Neurology Research (Friedsam Foundation).

[1] Strauss, H., and Landis, C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 369.

Following the injection of metrazol a seizure may or may not occur. Failure of an overt seizure to appear may be characterized by any one of the following EEG reactions: 1. Drop in the percent time alpha without the appearance of abnormal potentials and return to normal activity in a few seconds. 2. Appearance of abnormal potentials (potentials with a frequency of 3-6 cycles per second) with a slow return to normal after more than 45 minutes. 3. Larval seizure (outburst of a serial sequence of 3-6 cycle per second potentials) with a return to normal activity in less than 15 minutes.

The occurrence of an overt seizure is characterized by the following type of EEG activity:

1. During the latent period and through to the end of the second clonic stage there is continuous activity of a frequency of 30 cycles per second in the precentral region. Activity of a frequency of 10 cycles per second may be recorded from occipital leads during the latent period and the tonic stage. Occasionally activity of a frequency of 15-16 and 3-6 cycles per second may occur in motor-occipital leads during the latent period.
2. The post-convulsive relaxation period shows very low voltage random activity.
3. The recovery period is marked by a gradual increase in frequency from a predominating activity of 3 cycles per second to an activity of 6 cycles per second and finally a complete restoration of normal activity.

A prolonged reaction to the metrazol seizure has been observed. Immediately following the seizure the patient exhibited a disorientation as to time, person and place, which was accompanied in the EEG record by an almost continuous activity of a frequency of 3 cycles per second. The EEG showed a gradual return to normal after one week. This was accompanied by a similar clinical improvement.

10285 P

Prenatal Intestinal Movements in Anoxemia.

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The extent to which fetal gastro-intestinal movements occur normally in intrauterine life is unknown. However, we are led to believe that they are present because meconium passed at birth contains vernix caseosum derived from the amniotic fluid which the human fetus swallows in large amounts by the sixth month.[1, 2] Carlson and Ginsburg[3] recorded vigorous and frequent hunger contractions in puppies delivered 8-10 days prematurely and direct observations of "spontaneous" fetal intestinal movements have been made by Yanase,[4] and others.[5-9] Asphyxial conditions prevailed in the latter instances except when viable fetuses near term were used.

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* Aided by a research grant from the American Academy of Arts and Sciences.

We have observed directly and recorded in motion pictures intestinal activities in fetal cats and in kittens one and 2 days after birth. Fetuses were delivered by Caesarian section performed on animals previously decerebrated under ether by tying internal carotid and basilar arteries. This made it possible to dispense with anesthesia. Fetuses were first examined with the placental circulation intact in a constant temperature bath of Locke's solution. Later the umbilical cord was clamped or the placenta disengaged from the uterus. Local procain anesthesia was used for the young kittens. We tried to evaluate the part played by anoxemia in influencing fetal gut motility and had available blood-oxygen determinations made in the same specimens and in litter mates as well as in the mother cats by the Van Slyke and Neill method.

Local constrictions of the intestinal wall were induced by mechanical and faradic stimulation in cat embryos 18.5 mm long weighing about a gram. "Spontaneous" movements were seen in specimens of 35 mm (3 g) at about the middle of gestation. Strength of intestinal movements increased rapidly from then on and by 70 mm (18 g) the behavior of the stomach and intestines appeared to be no different from that in unanesthetized kittens a day or two old (110-120 g).

All specimens studied were subject to some anoxemia under the conditions of the experiments even though the placentas were intact and umbilical vein blood was fairly red. Approximately 50-60% saturation with oxygen (8-9 vol %) was never exceeded in the umbilical vein blood.[10] It was determined that rhythmical fetal

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[1] de Snoo, K., *Monatschr. f. Geburtsh. u. Gynäk.*, 1937, **105**, 88.

[2] Ehrhard, K., *München. med. Wchnschr.*, 1937, **84**, 1699.

[3] Carlson, A. J., and Ginsburg, H., *Am. J. Physiol.*, 1915, **38**, 29.

[4] Yanase, J., *Pflüger's Arch.*, 1907, **117**, 345; *ibid.*, 1907, **119**, 451.

[5] Preyer, W., *Specielle Physiologie des Embryo*, 1885.

[6] Friedman, M. H. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 495.

[7] Tani, K., *Jap. J. Obstet. and Gynec.*, 1927, **10**, 2.

[8] Inada, N., *Fukuoka Acta Med.*, 1928, **21**, 596.

[9] Koshtoyantz, C. S., and Mitropolitanakaya, R. L., *Fiziol. Z.*, 1934, **17**, 1309.

[10] Steele, A. G., and Windle, W. F., *J. Physiol.*, 1939, in press.

respiratory movements occurred when the average oxygen content was 6.5 volumes %; these specimens showed active digestive type of peristalsis. It was found, on the other hand, that fetuses were apneic when the average oxygen content was 2.1 volumes %; the amount of propagation in intestines tended to decrease in apneic fetuses but rhythmical segmentation persisted. It would seem, then, that fairly normal intestinal motility is compatible with marked anoxia in cat fetuses. That gut movements would have been as vigorous and frequent or that they would have occurred at all had better oxygenation prevailed we cannot say.

When the fetuses were deeply depressed and in the asphyxia following interruption of the placental circulation, the tonus of the intestines diminished and agonal, pendulous, writhing movements appeared. Similar activity was induced under profound asphyxia in the newborn kittens.

10286 P

Cure of Microcytic Hypochromic Anemia in Dogs with Crystalline "Factor I."

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Severe microcytic hypochromic anemia developed[1] when puppies were fed a purified casein diet apparently deficient only in factor I (rat antidermatitis factor, vitamin B_6). This anemia was cured by addition to the diet of a concentrate prepared from rice bran containing the missing factor. Knowledge at that time did not justify the conclusion that the material in rice bran extract which cures rat dermatitis is identical with the substance that cures anemia in dogs. This has now been shown to be the case by use of crystalline factor I.

The basic diet of 3 adult dogs[1] was supplemented daily with 0.05 mg of thiamine per kilo, 60 micrograms of crystalline riboflavin per kilo, 4.0 cc of liver extract 1A containing factor II (chick antidermatitis factor) per kilo,[1] 15 mg nicotinic acid, and halibut liver oil. When started on the diet, the dogs' red blood cell counts were 8.68, 6.88, and 7.53 million, hemoglobin 16.7, 12.3, and 16.2 g per 100 cc. Hematocrit of dogs 1 and 3 at beginning of study was 62 and 56 cc per 100 cc of blood respectively, while the mean corpuscular volume was 71.4 and 74.3 cubic micra. Microcytic hypochromic anemia developed in all 3 animals. Loss of appetite, weight, and severe constipation also occurred. Daily addition of 0.5 g of iron and ammonium citrate and 0.5 mg of copper sulphate to diet of 2 dogs failed to prevent increase in the anemia.

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[1] Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., *J. Nutrition*, 1938, **16**, 197.

The dogs were maintained on the diet until moderately severe anemia was present. Crystalline factor I prepared by the method of Lepkovsky*[2] was then administered by mouth in daily doses of 60 micrograms per kilo. The hemoglobin of the dogs was 6.2, 4.8, and 7 g, and the red blood cell counts 4.53, 3.54, and 5.29 million when therapy was started. Hematocrit of dog 1 was 25 cc, mean corpuscular volume 48.4 cubic micra 7 days before starting therapy. Hematocrit of dog 2 was 20 cc and mean corpuscular volume 54.5 cubic micra 3 days before starting therapy, while hematocrit of dog 3 was 27 cc and mean corpuscular volume 51 cubic micra on the day therapy was begun. Reticulocytes rose from 1.8, 2.3, and 0.0% to 14, 12.8, and 6.5% by third or fourth day of treatment, and this was followed by a rise in red cells and hemoglobin and increase in hematocrit and mean corpuscular volume. In 28 days the red blood cell count of dog 1 was 7.09 million, hemoglobin 14.5 g, hematocrit 44 cc and mean corpuscular volume 62.1. After 16 days on therapy the red blood cell count of dog 2 was 6.46 million, hemoglobin 14.2 g, hematocrit 43 cc and mean corpuscular volume 66.6 cubic micra. On the nineteenth day of therapy the red blood cell count of dog 3 was 7.61 million, hemoglobin 14.7 g, hematocrit 47 cc and mean corpuscular volume 61.8 cubic micra. The hematological improvement was associated with improvement in general condition of dogs, increase in appetite and gain in weight.

Conclusions. A dietary microcytic hypochromic anemia of dogs was cured by addition of crystalline factor I to the diet. These results indicate that the material (factor I) which cures rat dermatitis is identical with that which cures microcytic hypochromic anemia in dogs.

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* Aided with materials and use of equipment by the Vitab Corporation, Emeryville, Calif.; by Merck and Company and Eli Lilly and Company with materials and financial grants; and by WPA with materials and personnel under project A:P No. 465-03-3-209.

[2] Lepkovsky, Samuel, *J. Biol. Chem.*, 1938, **124**, 125.

10287 P

Effect of External Secretion of Pancreas upon Blood Lipids of Completely Depancreatized Dog Maintained with Insulin.*

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Previous reports[1, 2] demonstrated that pancreas contains a factor capable of increasing the blood lipids of the completely depancreatized dog maintained with insulin. It was shown that, following the ingestion of raw glandular tissue, the lipid levels rose well above normal and that all constituents were involved (cholesterol, phospholipids and neutral fat). The most striking change occurred in blood cholesterol, particularly in its esterified fraction. It was also established that this factor is heat labile and therefore not identical with the heat stable factor of the pancreas, which latter, when fed to depancreatized dogs maintained with insulin, prevents the infiltration of fat in their livers.[3] Furthermore, the blood lipid factor is not choline, for, although this can restore the blood lipid levels to normal, it fails to raise them appreciably above normal.[4]

The studies just described were made by adding whole pancreas to an otherwise adequate diet. It seemed important to determine whether similar results could be obtained by substituting the external secretion of the pancreas for the whole gland. The work reported here shows that the pancreatic juice does contain a factor which affects in a similar manner the blood lipid levels of the depancreatized dog maintained with insulin.

Pure pancreatic juice was collected by means of a modified Elman-McCaughan[5] fistula from dogs that were kept in good nutritional state by the feeding of an adequate diet containing 30 to 50 g of raw pancreas daily and whose salt balance was maintained through replacement either by intravenous administration or by addition to the diet.

The depancreatized dogs were cared for as recorded elsewhere[3]

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* This work has been conducted in part under grants from the Christine Breon Fund for Medical Research. Assistance was also furnished by the Works Progress Administration.

[1] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

[2] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1935, **112**, 155.

[3] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **119**, 435.

[4] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **120**, 647.

[5] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

and were maintained on a diet consisting of raw lean meat, sucrose, bone ash and vitamin supplements. Feedings were given twice daily. Four experiments were performed on 3 animals. It already has been shown that, when raw pancreas is excluded from the diet of such animals, the blood lipid levels fall below normal. This exclusion was practiced in the 4 experiments, and as soon as the subnormal lipid levels were established, fresh pancreatic juice was added to the diet for a period of 3 weeks in amounts varying from 300 to 900 cc daily. When the pancreatic juice was mixed with other food, all the animals rapidly ingested the diet. This favorable result may be due to the fact that only dogs with a good appetite were chosen for the experiment.

All lipid constituents responded to the feeding of pancreatic juice by rising to levels above normal. The increases observed were comparable to those produced by supplementing the diet with 250 g of raw pancreas daily. The changes in the total, free and ester chol-

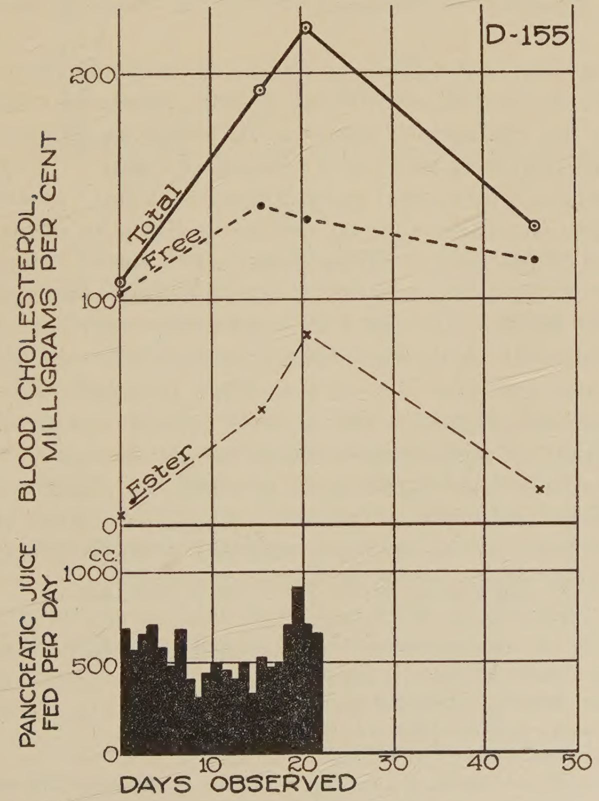

Fig. 1.

esterol in one experiment are shown in Fig. 1. The effects produced on cholesterol esters are especially striking. As shown in the chart, this lipid constituent rose from 5 to 81 mg %. Experiments to determine the nature of the substance that produces these lipid changes are in progress.

Summary. The external secretion of the pancreas contains a substance that causes a marked rise above the normal in the blood lipid levels of the depancreatized dog maintained with insulin.

10288

Influence of K-Strophanthosid on Elasticity of the Tortoise Ventricle.

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There has been uncertainty as to whether digitalis glucosides act to decrease the diastolic ventricular volume mainly by virtue of an increase in the efficiency of doing work[1] or by an increase in elasticity, frequently referred to as an increased tonus.[2] This problem has been attacked by several investigators. Cushny[3] misinterpreted his own data in stating that no change in elasticity occurred with large doses of glucoside. Other observers[4, 5] found either little or no change in elasticity with small doses of digitalis which were adequate to increase the force of contraction, and very large increases in elasticity with doses causing contracture. Working with strips of frog ventricle[6] and cat's papillary muscle,[7] other workers have found that great increases in work capacity occur with doses of drug which are without influence on resting elasticity.

We have performed experiments in which the diastolic and systolic elasticity and work of the tortoise ventricle were measured, and the influence of the cardiac glucoside K-Strophanthosid* upon

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[1] Peters, H. C., and Visscher, M. B., *Am. Heart J.*, 1936, **11**, 273.

[2] Cohn, E. J., and Steele, M., *J. Clin. Invest.*, 1932, **11**, 871.

[3] Cushny, A. R., *The Action and Uses in Medicine of Digitalis and Its Allies*, Longmans Green & Co., London, 1925, fig. 15.

[4] Eismayer, *Ergeb. d. Physiol.*, 1930, **30**, 126.

[5] Sulzer, R., *Z. f. Biol.*, 1932, **74**, 571.

[6] Ueda, S., *Acta Scholæ Med. Univ. Kioto*, 1924, **6**, 193.

[7] Cattell, J. M., and Gold, H., *J. Pharm. and Exp. Therap.*, 1938, **62**, 116.

* We are indebted to the Sandoz Chemical Works, Inc., for supplies of this and other material.

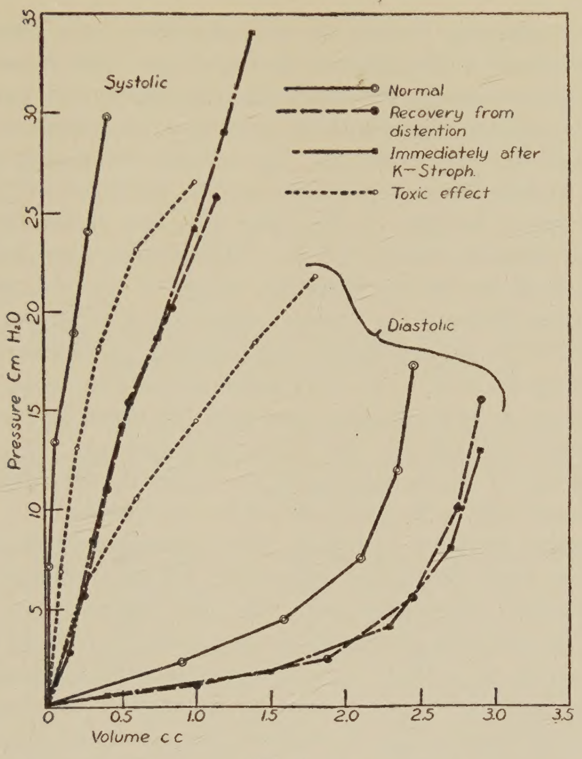

FIG. 1.

these factors. The method of measurement was essentially that employed previously,[8] and consisted in a device for measuring ventricular volume at various pressures in the normally beating ventricle in systole and diastole. Graded doses of the drug were used in different experiments.

The general result of all experiments is seen in Fig. 1, in which is shown the time course of changes following addition of 0.036 mg K-Strophanthosid to 180 cc of perfusion fluid, making a concentration of 1 part in 5 million. The observed changes are: (a) There is no change in diastolic elasticity over the first 10 minutes, while there was a great increase in work performed and tension set up. The pulse pressure at 2 cc diastolic volume before the drug was 10 cm H_2O and after it was 19 cm H_2O, an increase of 90%. The work performed increased in proportion. In experiments in which smaller doses were administered no further change occurred, other than the progressive decline in elasticity and work over time, reported elsewhere[8] in the absence of any drug. (b) However, when relatively large doses of glucoside were administered, (greater than 1 part in 7 million) the ventricle showed a progressive increase in diastolic and systolic elasticity, the former increasing more than the latter, so that the pulse pressure and the work fell off greatly. In this experiment, in curve 4 the pulse pressure diminished to 4 cm H_2O at the diastolic volume 1.8 cc. This is to be compared with the 19 cm H_2O figure during the early stage of the glucoside action. All gradations between the 2 occur during the toxic phase, and under the influence of varying doses. In complete contracture the pulse pressure became zero. (c) The most important result noted was that whenever a dose of drug increased the diastolic elasticity, the pulse pressure fell off and the work done declined. The increased work capacity of the heart muscle produced by this glucoside was not associated with a change in elasticity (or tonus).

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[8] Kabat, H., and Visscher, M. B., 1939, Submitted for publication in the *American Journal of Physiology.*

The further positive fact that, when increases in elasticity were produced by toxic doses, the work done fell off, indicates decisively that the theory, previously held, that the improvement in cardiac efficiency by digitalis glucosides is due even in part to changes in diastolic tonus, is without critical experimental foundation. This theory has been developed from experiments on mammalian heart where it is extremely difficult to dissociate effects of increased work capacity from direct effects upon elasticity. Improved oxygen supply to the myocardium increases elasticity directly[8] and since digitalis increases efficiency of doing work,[1] the oxygen balance is improved by it. An increase in ventricular elasticity in the failing heart after digitalis therefore need not be due to an effect on the physical property of elasticity by the drug, at all, but could be due simply to the indirect effect of improved work capacity. It is obvious that these two effects must be dissociated, which is accomplished readily in the case of the tortoise ventricle.

Summary. The cardiac glucoside K-Strophanthosid has no effect on the diastolic elasticity of tortoise ventricle with doses and within times which result in very large increases in tension set up and work done. Larger doses produce after a time great increases in diastolic elasticity, up to the point of contracture, but when such increases occur the work and tension production fall off. The increase in work capacity of tortoise ventricular muscle produced by this glucoside is not positively correlated with diastolic elasticity changes.

Inferences from observations on the failing mammalian heart are confused by the fact that anoxia itself alters elasticity, and therefore the increased efficiency produced by digitalis would in itself be expected to increase diastolic elasticity. Such an indirect effect is a secondary result of the primary action of the drug upon work performance.

10289

Liver Regeneration in Rats Protected with Xanthine Against Carbon Tetrachloride Poisoning.

O. GARTH FITZHUGH. (Introduced by P. D. Lamson.)

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Many investigators have attempted to prevent liver necrosis which may arise from the industrial or medical use of carbon tetrachloride or chloroform. Sato[1] described a liver hormone, "Yakriton," prepared from ox livers. Since that time Sato and his collaborators, in a series of nearly a hundred papers, have reported excellent results from the use of this detoxicating hormone. Among the more important claims for "Yakriton" has been the prevention of chloroform necrosis. Recently, Forbes and Neale[2] and Forbes, Neale, and Scherer[3] prepared a liquid extract of hog livers, which, when administered to albino rats prior to acute poisoning with carbon tetrachloride or chloroform, gave a protective action against these drugs. This protective agent was later crystallized from the crude extract by Forbes and McConnell.[4] Neale and Winter,[5] continuing these investigations, were able to identify as xanthine or sodium xanthine the crystalline material that protected against carbon tetrachloride and chloroform. The protective action of liver extract, as well as of sodium xanthine, has been further confirmed by Barrett, MacLean, and McHenry.[6]

Because of the observations made in this laboratory on calcium as a protective agent in carbon tetrachloride intoxication,[7] we decided to study further the action of xanthine in carbon tetrachloride poisoning. The large dose of xanthine required and the insolubility of this substance led us to use a method similar to that of Neale and Winter.[5] During our study of the microscopic sections from the livers of rats protected with xanthine, certain points of interest were noted. The livers of rats protected with xanthine and killed 24 hours after the administration of carbon tetrachloride showed little or no protection in comparison with the controls. At the 48-hour period, however, a marked difference could be seen in the 2 groups of sections. The variation in extent of necrosis in the livers from the xanthine-treated rats killed 24 hours after the administration of carbon tetrachloride prevented any conclusion that repair had occurred between the 24- and the 48-hour periods. Nevertheless, we did see mitotic figures in some liver sections from the xanthine-treated rats killed 48 hours after the administration of carbon tetrachloride. These facts led us to consider the possibility of a stimulated regeneration of the liver cells by xanthine.

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[1] Sato, A., *Tohoku J. Exp. Med.*, 1926, **8**, 232.

[2] Forbes, J. C., and Neale, R. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 319.

[3] Forbes, J. C., Neale, R. C., and Scherer, J. H., *J. Pharm. and Exp. Therap.*, 1936, **58**, 402.

[4] Forbes, J. C., and McConnell, J. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 359.

[5] Neale, R. C., and Winter, H. C., *J. Pharm. and Exp. Therap.*, 1938, **62**, 127.

[6] Barrett, H. M., MacLean, D. L., and McHenry, E. W., *J. Pharm. and Exp. Therap.*, 1938, **64**, 131.

Experimental. Xanthine protection. Male albino rats, weighing from 125 to 200 g, were used in all experiments. The diet employed was either the commercially prepared "Purina Chow" or the mixture used by Forbes, Neale, and Scherer[3] of yellow corn meal 35, wheat 12, oats 10, wheat bran 5, wheat germ meal 5, alfalfa 5, dried skim milk 15, dried meat scraps 10, calcium carbonate 1.5, sodium chloride 0.5, and yeast 1%. All animals were fed one of these 2 diets from 10 days to 2 weeks before the experiment began. Several groups of rats were allowed no food for a period of 24 hours prior to the administration of carbon tetrachloride. The relative results were affected neither by the diet nor by the withdrawal of food.

One subcutaneous injection of 100 mg of xanthine per 100 g of body weight was made 24 hours before the administration of carbon tetrachloride. A finely ground, aqueous suspension of xanthine was employed. The carbon tetrachloride was given orally by means of a stomach tube, in doses of 2, 3, or 4 cc per kg of body weight. The rats were killed at intervals of 24 or 48 hours after the administration of carbon tetrachloride. The experimental groups of animals consisted of 157 rats given 4 cc, 103 given 3 cc, and 82 given 2 cc of carbon tetrachloride per kg of body weight (Table I). Small slices of the livers of all rats which survived for the duration of the experiment were fixed in Bouin's fluid. Paraffin sections were cut to 5 microns in thickness and stained with hematoxylin and eosin. In all cases the same lobe of the liver was used for microscopic examination to insure uniformity of the sections.

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[7] Minot, A. S., *J. Pharm. and Exp. Therap.*, 1931, **43**, 295.

Our experience with the xanthine was essentially the same as that reported by Neale and Winter.[5] It gave a relative protection to the livers against the central necrosis caused by carbon tetrachloride. We were able to observe a difference between the livers of the rats that had had an injection of xanthine prior to the administration of carbon tetrachloride and of those that had received no xanthine. Sections of livers from the control animals killed at 48 hours exhibited the typical central necrosis with its fatty and round cell infiltration extending toward the periphery of the lobule, in some cases involving 50 to 80% of the liver lobule. Although sections of livers from the xanthine-treated rats killed at the corresponding period showed some damage, the extent of the necrosis was significant. In the latter the necrosis, in most cases, was limited to a small area around the central lobular vein, and the remainder of the lobule appeared normal.

TABLE I.
Mortality of Rats Given Carbon Tetrachloride and Xanthine.

Dose of CCl_4 (cc/kilo body wt)	Dose of Xanthine (mg/100 g body wt)	Time observed after CCl_4 (hr)	No. dead	No. alive	% survived
4	100	24	22	16	42.1
	0	24	24	14	36.8
	100	48	9	5	35.7
	0	48	44	23	34.3
3	100	24	1	22	95.6
	0	24	11	15	57.2
	100	48	5	10	66.7
	0	48	19	20	51.3
2	100	24	0	20	100.0
	0	24	0	15	100.0
	100	48	1	30	96.8
	0	48	1	15	93.8

The mortality rates of the controls and the xanthine-treated animals were not materially different except in those that received 3 cc of carbon tetrachloride per kg (Table I). In this group 30 from a total of 65 rats used as controls died, and of the 38 that received xanthine only 6 died. The mortality of the rats receiving 4 cc of carbon tetrachloride was more than 50% in both cases, whereas of those that received 2 cc only one died in each group. From a mortality standpoint, the xanthine may have protected the rats that received a moderately toxic dose. A larger amount of xanthine was not administered because much of the 100 mg per 100 g of body weight remained unabsorbed at the site of injection.

Cell regeneration. Since colchicine has been shown to stop cell division in the metaphase,[8] we used this substance to determine the growth rate of the liver cells. The rats were given the moderately toxic dose of 3 cc carbon tetrachloride per kg of body weight. Colchicine, in the amount of 0.15 mg per 100 g of body weight was injected subcutaneously 9 hours before the time of sacrificing the animals. The xanthine was administered in the manner described previously. Animals were killed 24, 36, 48, 72, and 96 hours from the time of carbon tetrachloride administration. Livers from 96 rats, about equally divided between xanthine-treated and controls, were studied. Microscopic slides of the livers were prepared with the same technic that was used with the first series.

TABLE II.
Percentage of Hepatic Cells in Mitosis.

Time after CCl_4 administration (hr)	No. of rats	Cells in Mitosis (%)	
		Xanthine-treated	Controls
24	18	0.21	0.20
36	25	0.32	0.29
48	22	4.70	6.60
72	16	1.30	1.71
96	15	0.25	0.20

Mitoses were counted under the oil immersion objective and over an area containing approximately 1000 hepatic cell nuclei. At least 500 nuclei were actually counted, and, in sections of even thickness, other measured areas of the same size were assumed to contain the same number of cells. Only areas with normal-appearing cell nuclei were selected for counting. The number of cells in mitosis is expressed in Table II as the percent of hepatic cell nuclei dividing in a given area. It is assumed (within the reasonable limits of error) the same number of cell nuclei would be dividing in other selected areas.

No difference in the number of mitotic figures in the least damaged areas of the livers could be observed. The necrotic areas had no cells in division. Fewer areas appeared normal in the controls; consequently, numerically there were more mitotic figures in the liver sections from the rats that had received xanthine. However, in like areas the number of cells appearing in the metaphase was not significantly different.

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[8] Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **18**, 411.

Mitotic figures in the livers of the xanthine-treated rats and of the controls for the 24- and 36-hour periods averaged only from 2 to 4 per thousand hepatic cells. This was, however, a greater number than has been observed in normal rat livers.[9] The greatest cell division occurred at the 48-hour period. Mitoses at this time averaged 47 and 66 per thousand hepatic cells for the livers from the xanthine-treated and from the controls, respectively. However, there was extreme variability in the number of mitotic figures at the 48-hour period. In the livers from the xanthine-treated rats the mitotic rate varied from 6 to 127 per thousand, and in the livers from the controls the rate likewise varied from 11 to 124 per thousand.

The rate of cell regeneration in the livers of the rats killed 72 hours after the administration of carbon tetrachloride was less than one-third the rate of those at the 48-hour period. At the 72-hour period the extent of the damaged areas had become considerably decreased, and there was no longer the extreme need for so rapid repair. Although complete recovery in the liver lobule had not occurred in 96 hours, mitosis had returned to a low rate.

Summary and Conclusions. Our experiments confirm the observations of Neale and Winter[5] that xanthine has a protective action against carbon tetrachloride poisoning. The extent of the necrosis in the liver lobule is decreased by xanthine. Liver protection was observed best in rats killed 48 hours after the administration of carbon tetrachloride. At this time the damage in the livers from the xanthine-treated animals was limited to a small area around the central lobular vein, whereas in the controls the necrotic area involved from 50 to 80% of the liver lobule.

Xanthine given prior to a moderately toxic dose of carbon tetrachloride lowered the mortality rate. When large doses of carbon tetrachloride were administered, no difference in the mortality rate was observed.

We observed no evidence that xanthine stimulated the regeneration of cells in the damaged livers. The number of mitotic figures in the livers of the xanthine-treated rats did not differ significantly from the number in the livers of the controls.

Mitosis increased in rate from the 24-hour period to the 48-hour period. At the 48-hour period it was occurring rapidly in the livers of both the controls and the xanthine-treated rats. After 48 hours the number of mitotic figures per thousand hepatic cells decreased. By 96 hours relatively few cells were in mitosis.

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[9] Brues, A. M., and Marble, B. B., *J. Exp. Med.*, 1937, **65**, 15.

10290

Protoplasmic Movement in Cardiac Muscle During Interval Between Contractions.

Charles M. Goss.

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The question of whether muscular contraction is related to the more general type of protoplasmic motion exhibited by nearly all cells is brought forward and partially answered by the following observations of both types of motion in the same cells.

The material consisted of tissue cultures of cardiac muscle whose spontaneous rhythmic contractions were interrupted by periods of rest. The explants were taken from the hearts of 16-day-old rat embryos and cultivated in a medium of rat plasma and embryo extract.[1] Most of the observations were with cultures incubated for 4 or 5 days without subculturing. The spontaneous contraction of heart muscle in tissue cultures is subject to variation both in the rapidity of its rate and the regularity of its rhythm. One of the irregularities is a remittent form in which short periods of rhythmic contraction alternate with similar or longer periods of rest. These cultures with remittent activity provided material in which cells of known contractile power could be studied during periods of quiescence.

The strands of new muscle growth which were to be observed for the slow unspecialized protoplasmic motion were first watched through the microscope to make sure that all the muscular elements contracted vigorously during the period of rhythmic activity. They were then focused on the film of a 16 mm movie camera through the 1.5 mm, 120× objective of the microscope. In order that the slow protoplasmic motion might be appreciated by the eye, it was made more rapid on the film by increasing the time between exposures. The exposures were taken every 5 seconds. Projected at the rate of 16 frames per second, the finished film increased the rapidity of motion 80 times.

In these pictures the outlines of the nuclei and the nucleoli were clearly visible. The cytoplasm contained compactly arranged and evenly distributed rod-shaped and granular bodies which have been identified as mitochondria.[2] Occasional fat droplets were recognized by their brilliant refractivity.

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[1] Goss, C. M., *Arch. f. exp. Zellforsch.*, 1933, **14**, 175.

[2] Stilwell, E. F., *Arch. f. exp. Zellforsch.*, 1938, **21**, 446.

In the speeding up produced by the cinematographs, the interior of the muscle appeared to be a seething mass. Near the nucleus, the mitochondria were jostled against each other in a manner as irregular as Brownian motion. Toward the ends of the elongated fibers, the motion was more restricted. A typical journey for a granule in this portion consisted of a halting progress for some distance in the direction of the long axis of the fiber and back again. Motion in a direction perpendicular to the long axis was less frequent. The term "cell" has been avoided as much as possible because the outlines of the individual elements of the muscle were seldom visible. The presence of cell boundaries was indicated in certain places, however, by narrow zones across which the mitochondria did not pass.

Photographs were taken at 5 second intervals during the periods of rhythmic contraction as well as during the periods of rest. The record of slow protoplasmic motion was interrupted and confused by the succession of rapid movements of the entire fibers. In order to check the protoplasmic motion during the interval between each contraction of a regular rhythm, films of cultures with slow regular rhythms were taken at a more rapid exposure rate. They indicated that the slow type of motion was present during the interval.

The protoplasmic motion cannot be confused with the rapid movement of contraction. The former is so slow that it must be made more rapid by artificial means, as described above. The latter, on the other hand, is so rapid that it must be slowed. A description of slow-motion cinematographs of contracting muscle has been published,[3] and is unnecessary here. The protoplasmic motion described above is distinct from the quivering of the entire fiber which follows the full contractions.[3] It has no relation to the independent contraction of individual cells, or to the microfibrillation of Fredericq which Murray[4] has given the suggestive name of "twittering."

It has been stated that most of the observations were made with 4- or 5-day cultures. In these, the muscle was embryonic or undifferentiated. Similar pictures were taken of older cultures in which the muscle had reached the earlier stages of differentiation characterized by an arrangement of the mitochondria into transverse rows resembling cross striations.[1] Here the protoplasmic motion was less extensive and the individual mitochondria made more restricted excursions.

The above observations indicate that the slow generalized motion is distinct from the movement of muscular contraction. It is associated, apparently, with a physical state common in all forms of protoplasm. Muscular contraction, on the other hand, is probably associated with a different physico-chemical system superimposed upon the other.

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[3] Goss, C. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 292.

[4] Murray, P. D. F., *Proc. Roy. Soc.*, Ser. B, 1934, **115**, 380.

10291 P

Intramuscular Administration of Anti-Pneumococcal Serum in Infants and Children.

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The intramuscular administration of a single dose of antipneumococcal serum in the treatment of pneumonia in childhood was attempted in an effort to obtain the advantages of serum-therapy without the dangers and technical difficulty inherent in intravenous administration. A very severe reaction with a marked chill and a rise in temperature to 108.6° followed the intravenous administration of serum to a child 7 years of age ill with Type I lobar pneumonia, and although she recovered we did not feel that in a disease which carried such a low mortality we were justified in jeopardizing the patient's chances of recovery. Nemir[1] reports 2 deaths which she believes were due to the intravenous administration of the serum. The above experiences with the intravenous route are not uncommon in institutions where a great deal of serum-therapy is used. Without serum-therapy the mortality varies from 4 to 7%. Trask[2] reported a mortality of 6% in type specific pneumococcal pneumonias studied at New Haven. Holt and McIntosh[3] stated that of 1482 cases (over 3 years of age) taken from hospital practice the mortality was 4%. 497 cases (all ages) observed in our hospital from 1928 to 1936 showed a mortality of 7%.

Secretion for typing was obtained by means of a pharyngeal swab. In some of our cases, 24 to 48 hours elapsed before material containing pneumococci could be obtained for satisfactory typing.

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[1] Nemir, R. L., *J. Pediatrics*, 1938, **13**, 219.

[2] Trask, James D., O'Donovan, Charles, Jr., Moore, Dorothea M., and Beebe, Agnes, *J. Clin. Invest.*, 1930, **8**, 623.

[3] Lobar Pneumonia—Holt's Diseases of Infancy and Childhood, 10th Edition, p. 436.

TABLE I.

Infants—Age	Day of Disease	Lobe	Type	Serum	Amount	Result	C.
(4) 5 mo	2	R.U.	18	H	60,000	Crisis 24 hr	0
7 ,,	2	,,	14	R	100,000	Lysis 36 ,,	0
(3) 12 ,,	1	,,	14	R	100,000	Crisis 24 ,,	0
13 ,,	2	L.L.	14	R	100,000	,, 24 ,,	0
(1) 14 ,,	4	L.U.	7	H	60,000	Lysis 36 ,,	0
18 ,,	7	R.U.	14	H	60,000	Crisis 18 ,,	0
18 ,,	2	,,	14	R	60,000	,, 18 ,,	0
20 ,,	6	,,	14	R	100,000	,, 24 ,,	Empyema
(2) 21 ,,	4	,,	14	H	60,000	,, 18 ,,	0
23 ,,	3	,,	14	R	60,000	Lysis 36 ,,	0
Children—Age							
2½ yr	3	R.L.	14	R	140,000	Crisis 24 hr	0
2½ ,,	6	,,	14	R	80,000	,, 18 ,,	0
2½ ,,	4	R.U.	14	R	80,000	Lysis 30 ,,	0
3 ,,	2	L.L.	4-8	R	120,000 each	Crisis 24 ,,	0
3½ ,,	8	,,	11	R	100,000	Lysis 48 ,,	0
(20) 4 ,,	2	R.L.	5	R	120,000	Crisis 24 ,,	0
4½ ,,	3	,,	1	R	120,000	,, 12 ,,	0
5 ,,	3	L.L.	1	H	80,000	,, 24 ,,	0
(5) 5 ,,	6	,,	1	R	140,000	Lysis 48 ,,	0
(6) 5 ,,	5	R.L.	1	R	160,000	,, 48 ,,	0
6 ,,	8	R.U.	1	H	100,000	Crisis 24 ,,	0
(7) 6 ,,	5	R.L.	7	H	80,000	,, 18 ,,	0
7 ,,	4	L.U.	1	R	200,000	,, 24 ,,	0
10 ,,	4	L.L.	1	R	100,000	,, 24 ,,	0

Later, a special laryngeal cannula with a syringe attached was devised by one of us (V R.). This enabled us to get tracheal pneumococcus-containing sputum which in most instances was adequate for satisfactory typing at the first attempt.

Patients selected for treatment were those that appeared to be seriously ill; the milder cases were not treated. Half of the serum was administered into the muscle of each buttock. We used either rabbit or horse serum. If the child showed a positive ophthalmic or cutaneous reaction to horse serum, rabbit serum was used. However, not all the patients treated with rabbit serum were sensitive to horse serum.

Table I illustrates the cases treated and results obtained. It will be noted that in this series Type 14 predominates in the infants and Type I in the older children. The amount of serum used varied between 60,000 to 100,000 units for infants and 80,000 to 200,000 for the older children. The day of the disease when serum was administered varied from 1 to 8 days. The results of treatment were very encouraging. Seven of the infants had a critical drop in temperature and symptoms 12 to 24 hours after treatment and, in 3, the temperature subsided by lysis over a period of 24 to 48 hours. Ten of the older children had a critical drop in temperature and symptoms within 12 to 24 hours and 4 subsided by lysis in 24 to 48 hours. There were no deaths.

Figure 1 shows some of the temperature-curves typical of this series.

In Case 20 (a Type V pneumonia) the agglutinin-titer was determined before administration of serum and 4, 8, 12, and 48 hours

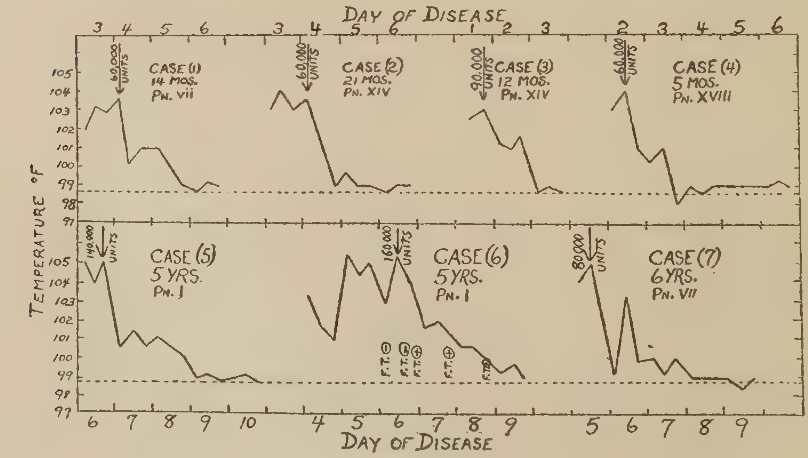

FIG. 1.

after administration of the serum. There was no agglutination present before administration of serum, but at the 4-hour period and the subsequent ones marked agglutination was demonstrable. In Case 6 (illustrated) reaction to the Francis test was negative 4 hours after administration of serum but 6 hours later it was strongly positive. These determinations would indicate that within a comparatively short time a sufficient amount of antipneumococcal antibody is absorbed from the muscle to account for the results obtained.

The Francis test or agglutinin-titration is now performed on all children who receive rabbit antipneumococcal serum and the agglutinin-titer is determined on all children who receive horse antipneumococcal serum.

We realize that this preliminary series is too small to draw final conclusions. However, the response to the intramuscular administration of antipneumococcal serum in 24 seriously ill children was decidedly favorable in all instances, resulting in a shorter course and in the recovery of all of them.

10292

Effect of Ether Peroxides in Wheat Germ Oil on Production of Tumors in Rats.

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Rowntree and associates[1] have reported a high incidence of abdominal sarcoma in Wistar rats, as well as albino rats from the Buffalo and Yale strains, when fed crude wheat germ oil prepared by ether extraction. Great variability occurred in the rate of tumor production. In rats given 1 cc of oil daily tumors appeared in about 100 to 200 days; with 4 cc daily the average was about 54 days. The nature of the neoplastic agent has not been determined but since it appears to be absent from refined ether-extracted wheat germ oil, and wheat germ oils not obtained by means of ether, it is possible that the factor is produced by some action of ether on wheat germ. Owing to the tendency of unsaturated fatty compounds to undergo oxidation while in contact with ether containing peroxides[2] it appeared to us that the neoplastic agent might have arisen through the action of such peroxides, formed in the extraction of wheat germ with ether, although the latter was reported to be peroxide-free before it was used.

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[1] Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359; Rowntree, L. G., Steinberg, A., and Brown, W. R., *Trans. Assn. Am. Physicians*, 1938, **53**, 199.

We have tested this hypothesis as follows: Two volumes of C. P. peroxide-free anhydrous ether were added to one volume of fresh cold pressed wheat germ oil* in a glass flask fitted with a condenser. This oil was aerated seven hours by means of a slow current of air drawn through H_2SO_4 and glass wool. The mixture was allowed to remain in the flask at room temperature for 36 hours, without protection from light, after which most of the ether was removed by distillation at atmospheric pressure. The mixture was then placed in an evaporating dish and heated on a water bath for 2 to 3 hours, with occasional stirring to remove most of the ether that remained. Following this the "etherized" oil was placed in a brown glass bottle and stored in the refrigerator. A faint trace of ether remained in it. The peroxide content, determined according to directions by Wheeler,[3] was 0.061 M of peroxide per 1000 cc of oil, whereas that of the "unetherized" oil was 0.030 M.

Eight McCollum strain rats, sexes evenly divided and weighing 45 to 55 g each, were used to test the etherized oil. Eight littermates similarly selected were used as controls, *i. e.,* given unetherized oil. All animals were kept in individual cages. During the first 117 days each animal was given 1 cc of the appropriate oil in supplement dishes, after the food pans had beeen removed. After the oil had been consumed the food pans were returned. Later the dosage of oil was increased to 2 cc per rat daily. Larger quantities could not be forced upon the animals without marked reduction in the food intake. McCollum's stock colony ration was used.

After 170 days all of the rats were killed and autopsied. No tumors were found and in all respects the 2 groups were identical.

This result does not constitute a failure to confirm the findings of Rowntree and associates since we employed cold pressed wheat germ oil, found by them to be without sarcogenic effect. It demonstrates, however, that the rat tumor-producing factor is not formed in cold pressed wheat germ oil through the action of ether peroxides, as determined in our rats over a period of time stated by Rowntree and associates to cause a high incidence of tumors in rats fed crude ether-extracted wheat germ oil.

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[2] Rieche, A., *Z. f. angew. Chem.*, 1931, **44**, 896.

* ADM wheat germ oil kindly furnished by the Archer-Daniels Midland Co., Minneapolis, Minn.

[3] Wheeler, D. H., *Oil and Soap*, 1932, **9**, 89; *Chem. Abst.*, 1932, **26**, 3128.

10293

Sulfanilamide in Treatment of Experimental *Shigella dysenteriae* (Shiga) Infections of Rabbits.

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Shigella dysenteriæ (Shiga) was cultivated on blood agar for 24 hours and suspended in sterile saline solution to give approximately 2000 million per cc. Six rabbits were inoculated intravenously with amounts varying from 0.05 cc (100 million) to 0.4 cc (800 million) per kilo of weight with the results shown in Table I.

TABLE I.
Virulence of *Shigella dysenteriæ* (Shiga) for Rabbits by Intravenous Inoculation.

No.	Dose suspension[*] per kilo (cc)	Equivalent in *Shigella*	Results in days 1	2	3	4	5
1	.05	100 million	—[†]	—	—	—	—
2	.1	200 ”	—	—	—	D	
3	.15	300 ”	—	D			
4	.2	400 ”	—	D			
5	.3	600 ”	D				
6	.4	800 ”	D				

[*] Suspension prepared of *Shigella dysenteriæ* (Shiga) suspended in saline solution from 24-hour blood agar cultures carrying 2000 million per cc.

[†] — = lived; D = died.

0.15 cc per kilo (300 million) proved fatal in about 40 to 48 hours.

Eighteen rabbits varying in weight from 1850 to 2380 g were inoculated intravenously with 0.15 cc suspension (300 million) per kilo of weight; 5 were untreated controls, all of which succumbed in 24 to 72 hours.

The remaining 13 were given sulfanilamide orally, subcutaneously and intravenously in the daily dosage per kilo (divided into 2 doses per day given 6 hours apart) shown in Table II for a total of 3 to 4 doses. The first dose was given 2 hours after intravenous inoculation with *Shigella dysenteriæ* (Shiga). All of the animals succumbed in 2 to 3 days after inoculation.

Three additional animals were used as drug controls; one received 0.5 g sulfanilamide orally per kilo per 24 hours, divided into 2 doses 6 hours apart, for 5 days (10 doses); one received 0.5 g per kilo subcutaneously and one 0.2 g per kilo intravenously, each being divided into 2 doses given 6 hours apart daily for 5 days (10 doses); all survived.

TABLE II.
Sulfanilamide in Treatment of Experimental *Shigella dysenteriæ* (Shiga) Infections of Rabbits.*

No.	Dose per kilo† (g)	Route of admin.	No. of doses	Results in days				
				1	2	3	4	5
1	.1	Orally	4	—‡	—	D		
2	.2	”	3	—	D			
3	.5	”	4	—	—	D		
4	.2	Subcut.	3	—	D			
5	.2	”	3	—	D			
6	.2	”	3	—	D			
7	.5	”	4	—	—	D		
8	.5	”	3	—	D			
9	.5	”	3	—	D			
10	.1	Intraven.	4	—	—	D		
11	.1	”	4	—	—	D		
12	.2	”	4	—	—	D		
13	.2	”	3	—	D			
14	Control	—	—	D				
15	”	—	—	—	D			
16	”	—	—	—	—	D		
17	”	—	—	—	D			
18	”	—	—	—	D			
19	.5	Orally control	10	—	—	—	—	—
20	.5	Subcutan. control	10	—	—	—	—	—
21	.2	Intraven. ”	10	—	—	—	—	—

*Inoculated intravenously with approximately 300 million per kilo.

†Divided into 2 doses daily given 6 hours apart; first dose given 2 hours after inoculation.

‡— = lived; D = died.

Conclusion. Sulfanilamide by oral, subcutaneous, and intravenous administration was ineffective in the treatment of rabbits inoculated intravenously with virulent *Shigella dysenteriæ* (Shiga) in dose of 300 million per kilo, fatal in 24 to 72 hours in untreated controls.

10294

Initiation of Lactation in the Albino Rat with Lactogen and Adrenal Cortical Hormone.

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The lactogenic hormone has been proved capable of initiating lactation in a number of animal species. In the rat, however, either slight or negative results have been reported. The early work has been summarized by Turner[1] and Nelson.[2]

Two possibilities might account for this lack of functional responsiveness: first, that the experimentally stimulated mammary glands of the rat are not developed to a state comparable to that which takes place during pregnancy and, secondly, that the pseudo-pregnant rat is non-responsive to lactogen.

Our previous observations,[3] however, demonstrated that secretory activity could be induced in the mammary glands of pseudo-pregnant rats by the injection of lactogen, pituitary glands from spayed rats, or glands from normal female rats. A fair amount of lactation was observed following the injection of pituitary glands from normal and ovariectomized rats which had been injected previously with estrogen. In not one instance, however, was the induced lactation comparable to that which occurs in the post-partum rat. Copious lactation was induced by injecting 30 mg of an acetone-dried powder made from cattle pituitaries into pseudo-pregnant rats which had been injected daily with 200 i.u. of estrogen during the pseudo-pregnant period.

Further attempts have been made to induce lactation in the pseudo-pregnant rat. Pseudo-pregnancy was induced by injecting 100 r.u. of a gonadotropic principle (Follutein†) into each of a group of multiparous rats.‡ The injection was repeated 6 days later. On the 12th day following the induction of pseudo-preg-

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[*] Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] Turner, C. W., *Sex and Internal Secretions*, 1932, chapter 12 (E. Allen, ed.), Williams and Wilkins Co., Baltimore.

[2] Nelson, W. O., *Physiol. Rev.*, 1936, **16**, 488.

[3] Reece, R. P., *J. Dairy Science*, 1938, **21**, 117.

† The Follutein and Prolactin used in this study were kindly supplied by Dr. John F. Anderson of E. R. Squibb and Sons.

‡ We are indebted to Dr. W. C. Russell for the rats employed in this work.

nancy the animals were ovariectomized and a mammary gland removed. Various hormone preparations were injected subcutaneously every 8 hours until 6 injections had been made. The acetone-dried powder (designated hereafter as A.D.P.) employed in this work was made from cattle pituitaries and suspended in distilled water so that 0.1 cc of the suspension contained 5 mg of the powder. The lactogenic hormone (Prolactin†) was in an aqueous solution of a pH 8 and contained 40 bird units (Riddle, Bates, and Dykshorn[4] unit) per cc. The adrenal cortical hormone (Eschatin) contained at least 25 dog units per cc. The dosages stated indicate total dosage. Three or 4 hours following the last injection the animals were sacrificed and their mammary glands observed macro- and microscopically. The mammary glands were rated according to the method used by Gardner and Turner.[5]

Nine control animals received the same treatment as the experimental animals with the exception of the injections following ovariectomy. The mammary glands of most of the control and experimental animals were well developed on the 12th day of pseudopregnancy. Secretory activity had begun in the mammary glands of 2 control animals at the time of ovariectomy. In the other 7 control animals there was no evidence of secretory activity. At the time of sacrifice the mammary glands of the control animals were rated (—). Histologic section showed, however, that secretory activity had been induced in the mammary glands of several of the control animals.

Three animals were injected with 30 mg of A.D.P. The mammary glands were rated (—). Two rats received 100 mg A.D.P. and 2 others 200 mg A.D.P. The mammary glands of both groups were rated (++).

Four animals were injected with 100 mg A.D.P. + 15 dog units of the adrenal cortical hormone and 4 additional animals were injected with 100 mg A.D.P. + 15 d.u. of the adrenal cortical hormone + 3.0 cc of a 20% glucose solution. The average rating of the mammary glands of these animals was (++).

Five rats received 100 bird units of the lactogenic hormone + 15 d.u. of the adrenal cortical hormone + 3.0 cc of a 20% glucose solution. The mammary glands of these animals were rated (++ to +++).

Five animals received 150 b.u. of lactogen + 24 d.u. of the adrenal cortical hormone. In this group the mammary glands were rated

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[4] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

[5] Gardner, W. U., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1933, 196.

(+++). The mammary glands had the appearance of being in full lactation and on section the alveoli and ducts were found to have been distended with milk.

Conclusions. It is possible to initiate lactation in the pseudo-pregnant rat by injecting lactogen + the adrenal cortical hormone or lactogen + the adrenal cortical hormone + a 20% glucose solution. It would seem, therefore, that the refractoriness of the mammary glands of the pseudo-pregnant rat to lactogen can be explained on the assumption that another hormone becomes the limiting factor in milk secretion before lactogen can fully express itself.

10295 P

Effect of Pectin on Bacterial Growth.

PAUL S. PRICKETT AND NORMAN J. MILLER. (Introduced by W. M. Cox, Jr.)

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Several years ago we became interested in pectin when it was suggested[1] that this substance appeared to exert a germicidal action on intestinal organisms. We have tried various technics for studying different concentrations of numerous commercial pectins of different grades at various H-ion concentrations. From these studies we have developed a testing technic that appears to be satisfactory.

It is necessary to test different pectins under the same conditions to obtain comparable results. Since the higher the concentration the more difficult it is to dissolve the pectin and since the more insoluble pectins are dissolved only by severe heat treatment, which tends to break down the pectin molecule, we have standardized on a 1% pectin solution in nutrient broth. However, we have tested the more soluble pectins at concentrations up to 5% with similar results.

Each pectin was dissolved with minimum heat treatment in sterile nutrient broth of pH 6.5. These pectin broths had low pH's, ranging from 5.5 to 3.9. At the "natural" pH thus obtained, we studied the growth of *Esch. coli.* Similar studies were conducted on each pectin broth adjusted to pH 6.5, which adjustment was made at room temperature to eliminate degradation of the pectin by heat and alkali. As controls for each pectin, we used nutrient broth adjusted to the corresponding "natural" pH and also nutrient broth adjusted to pH 6.5. All pH determinations were made with the glass electrode. One hundred cc each of these 4 broths were inoculated with 1 cc each of the same 24-hour broth culture of *Esch. coli,* and incubated at 37°C. Plate counts were made after 24 and 48 hours at this temperature.

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[1] Personal communication from Dr. C. A. Tompkins.

Results obtained from studying 13 different commercial pectins of widely varying grades and types showed that pectins *per se* do not exert germicidal action. This does not confirm previously reported findings.[2] In fact in all 13 pectin broths adjusted to pH 6.5 and also in the control broths at the same pH the inoculated *Esch. coli* showed large increases after 24 and 48 hours' incubation. Furthermore in the 9 pectin broths with "natural" pH's ranging from 5.5 to 4.9 and also in the correspondingly adjusted control broths the inocula showed large increases after incubation. On the other hand in the 4 pectin broths whose "natural" pH's ranged from 4.4 to 3.9 marked decreases of the inoculated *Esch. coli* occurred which were paralleled by the counts of the control broths adjusted to these same low pH's. Thus it is seen that H-ion concentration is the factor responsible for these decreases in counts. Additional confirmation of this conclusion is obtained from the pH of the pectin and control broths after incubation. Determination of this pH is necessary to interpret properly the data secured in this type of study. This is specially significant when studying pectins that contain fermentable carbohydrates as "filler", which are used frequently to standardize the grade of the pectin.

10296

Occurrence of Choline-Esterase in Swine.

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From the Laboratories of the Newark Beth Israel Hospital, Newark, N. J.

The purpose of this study was to find potent sources of choline-esterase which might be obtained conveniently in large amounts for studies on the purification and properties of this enzyme, and to make preliminary observations on its distribution within tissues of a single species in regard to its possible bearing upon nerve physiology.

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[2] Haynes, E., Tompkins, C. A., Washburn, G., Winters, M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 839.

* Aided by a grant from the Herbert L. Celler Foundation.

Heretofore, investigations have been carried out on the relative choline-esterase activities of a given organ[1] or body fluid[2, 3, 4] from various species, and a great variety of papers have appeared dealing with the activity of a certain tissue from a certain species, usually in connection with a definite physiological function. However, very little has appeared in the nature of a survey of the relative activities of the tissues within a given species.

Plattner and Hintner[5] determined, by pharmacological means, the time required for the destruction of 50% of the acetylcholine in digestion mixture by individual organ extracts or body fluids. A single species of animal was not used throughout as a source of material so that strict comparisons between different tissues was not always possible. Furthermore, the method of measurement employed was not very satisfactory since activity is no longer a linear function of time when even much less than 50% of the substrate is hydrolysed in a similar digestion mixture. Quastel, Tennenbaum, and Wheatley[6] measured the choline-esterase activity of slices of several tissues from the rat and guinea pig by the manometric chemical method, as well as the ability of these tissues to produce choline esters *in vitro*, and concluded that there is no correlation between the two. It is interesting to note that Shaw[7] has observed the acetylcholine concentrations in 4 organs of the dog to fall in the order: submaxillary gland > small intestine > stomach > liver, which happens to be the same as the order of the choline-esterase activities found in the present investigation for these organs in swine.

The organs from full-grown animals were removed within 1 hour after slaughtering and transported at once to the laboratory where they were minced in a meat grinder. Five g were weighed out and then subjected to further grinding in a mortar with washed and ignited sand. The disintegrated material was taken up with 10 ml bicarbonate-Ringer solution, centrifuged at a constant speed of about 2500 r.p.m. for 5 min., and the supernatant fluid decanted. In cases where the organ or tissue weighed less than 5 g the corresponding volumes of bicarbonate-Ringer solution were employed. When only small amounts of a tissue were available, or when the tissue could be easily disintegrated as in the case of brain, the initial mincing in the meat grinder was omitted. One-half ml of this fluid was used with 1.5 ml of substrate solution (containing 7.5 mg acetylcholine chloride dissolved in bicarbonate-Ringer solution) for the determination of the enzyme activity by the manometric procedure using the Warburg apparatus.[8] The thermostat was maintained at 30° and manometer readings were taken every 5 min for 30 min.

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[1] Marnay, A., *Compt. rend. soc. biol.*, 1938, **128**, 519.

[2] Stedman, E., Stedman, E., and White, A. C., *Biochem. J.*, 1933, **27**, 1055.

[3] Stedman, E., and Stedman, E., *Biochem. J.*, 1935, **29**, 2107.

[4] Hall, G. E., and Lucas, C. C., *J. Pharm. Exp. Therap.*, 1937, **61**, 10.

[5] Plattner, F., and Hintner, H., *Arch. ges. Physiol.*, 1930, **225**, 19.

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[7] Shaw, F. H., *Biochem. J.*, 1938, **32**, 1002.

Activity-time curves were constructed and only values of the linear initial reaction velocities were used. In cases of very great activity the course of the reaction was often no longer linear toward the end of the 30 min period so that it was necessary to extrapolate the linear portion to obtain the 30 min value.

A source of error in comparing the activities of the different tissues lies in the extraction procedure employed. In the ideal case, the enzyme would be completely removed from the tissues so that the extracts would enable comparisons of total activities. Previous experience has shown that choline esterase is readily extracted from small bits of tissue about equally as well by 30% glycerol or physiological saline solution.[9] However, it has been found in this work that a certain amount of the enzyme is retained by the residue after extraction, chiefly due to adsorption. The fineness to which the tissue is ground largely determines the completeness of extraction and since the particle-size undoubtedly varied in different grindings, a source of error was introduced at this point. In addition it has been observed for tissues very rich in choline-esterase that the total activities measured by the procedure described are somewhat less than those found when the extracts are used in greater dilution. In other words, the concentration of enzyme is so great in these cases that there is a deviation from the linear relationship between activity and enzyme concentration which holds for the lower concentrations. Nevertheless, for the purposes at hand the data obtained by employing a uniform extraction technic was sufficient to enable a fairly adequate comparison of tissue activities.

It has been observed that the extracts retain their activities quite well when stored in the refrigerator. Thus an extract of supracervical lymph nodes was found to have an activity corresponding to the liberation of 28 cmm CO_2 under the conditions employed in these experiments, and after 11 days the activity was unchanged. Another preparation changed from 35 to 31 in 18 days. Similarly an extract of submaxillary gland changed from 194 to 186 in 11 days, and fell to 168 after 7 more days. After 13 days several jejunal extracts remained unchanged.

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[8] Ammon, R., *Arch. ges. Physiol.*, 1933, **233**, 486.

[9] Glick, D., *J. Gen. Physiol.*, 1938, **21**, 297; *Compt. rend. lab. Carlsberg*, 1938, **21**, 269.

The data in Table I are self-explanatory. The lack of activity in bile and gall bladder is no doubt the result of inhibiting action of

TABLE I.
Choline-Esterase Activities of Various Swine Organs and Tissues.

Organ or tissue	Enzyme Activity[*] (mm^3 CO_2 evolved in 30 min. at pH 7.4 at 30°C by .5 ml extract)	Avg
Heart	23, 21, 24, 23, 29	24
Skeletal Muscle (Masseter)	25, 25, 26, 27, 26	26
Lung	20, 24, 28, 37	27
Liver	70, 77, 77, 74, 77	75
Spleen	64, 64, 52, 66, 61, 60, 56	60
Kidney	25, 17, 18, 18, 29	21
Pancreas	55, 60, 55, 55, 60	57
Thyroid	21, 23, 27, 32, 27	26
Thymus	30, 22, 29, 30	28
Adrenal	26, 28, 20, 26, 24	25
Testis	67, 67, 69	68
Ovary	45, 68, 53, 45	53
Fallopian Tubes	362, 256, 251, 352, 286	301
Endometrium	90, 83, 91, 87	88[‡]
Stomach Mucosa (Pylorus)	194, 186, 182	187[‡]
Duodenal Mucosa	182, 196, 160, 172, 159	174[‡]
Jejunal Mucosa	190, 198, 165, 200, 210	193[‡]
Lachrymal Gland	1032, 1320, 1056	1136[§]
Sublingual Gland (Mixed Mucous and Serous)	1200, 984, 786	990[§]
Parotid Gland (Serous)	2550, 2340, 2670, 2400, 2460, 2470	2481[§]
Submaxillary Gland (Mainly Mucous)	181, 220, 158, 223, 153	187
Cerebrum	45, 44, 48, 51, 60	50
Cerebellum	86, 85, 79, 99	87
Medulla Oblongata	130, 146, 163, 134, 117	138
Spinal Cord	81, 74, 81	79[‖]
Lymph Nodes (Mesenteric)	71, 53, 53, 56	58
” ” (Supracervical)	29, 21, 28, 35, 26	28
Lens	12, 6, 9, 15	11
Gallbladder Mucosa	0, 0, 0	0
Bile	0, 0	0
Serum[†]	9, 7, 7	8

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[*] A separate animal was used as the source of the material for each measurement upon any given organ or tissue.

[†] 0.5 ml of 10% serum used.

[‡] The mucosa was scraped off, and histological examination showed only traces of muscle tissue.

[§] Manometer readings taken every 2 min. until top of scale was reached.

[‖] Thoracic region.

the bile salts.[10, 11] One might expect the very high activity found in the salivary and lachrymal glands to be related to the fact that these tissues respond strongly to cholinergic stimulation.

Summary. The choline-esterase activities of various swine organs and tissues were measured, and particularly high activities were observed in the cases of the lachrymal and salivary glands, the fallopian tubes, alimentary mucosa, and the medulla oblongata.

The authors wish to thank E. S. Ring, D. V. M., and Mr. A. Jaejer of Swift and Co., Kearny, N. J., for their coöperation in supplying the animal material used in this investigation.

10297 P

Comparative Therapeutic Effects of Sulfapyridine in Experimental *Staphylococcus aureus* Infections in Mice.*

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It has been shown that sulfanilamide has a slight therapeutic effect in the treatment of experimental staphylococcal infections in mice.[1, 2, 3] However, its efficacy has not been great and the drug has been of little value in the treatment of staphylococcal infections in man, in which the tissues or blood stream have been seriously invaded by these organisms. Recently Whitby[4] without giving details of his experiments has shown that sulfapyridine has a definite chemotherapeutic effect in experimental staphylococcal infections in mice.

We have been testing the comparative therapeutic effects of sulfapyridine and sulfanilamide in experimental staphylococcus aureus

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[10] Sobotka, H., and Antopol, W., *Enzymologia*, 1937, **4**, 189.

[11] Antopol, W., Schifrin, A., and Tuchman, L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 363.

[*] This investigation has been supported by a grant from the Chemical Foundation, Inc.

[1] Buttle, G. A. H., *Proc. Roy. Soc. Med.*, 1937, **31**, 154.

[2] Mellon, R. R., Shinn, L. E., and McBroom, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 563.

[3] Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

[4] Whitby, L. E. H., *Lancet*, 1938, **2**, 1095.

TABLE I.

Comparative Therapeutic Effects of Sulfapyridine and Sulfanilamide in the Treatment of Experimental *Staphylococcus aureus* Infections in Mice.

No. of Mice	Drug	Size of Inoculum	Deaths—Day of Infection														Survivals 15 days
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
15	S.P.	780 Mil.						1			1		2	2		1	8
15	S.	” ”	2		2	1	1			1	1		2	1			4
10	Controls	” ”	2	5	3												0
15	S.P.	1.35 Bil.	1						1		2	2		1		4	4
15	S.	” ”		1	3	1	5	1	1			2	1				0
10	Controls	” ”		7		1	1	1									0
19	S.P.	16 Bil.	2	2	2			2	2		3		2				4
20	S.	” ”	7	1	3	1	1	2		2	1				1	1	0
10	Controls	” ”	4	4	2												0

S.P.—Sulfapyridine.
S.—Sulfanilamide.
Mil.—Millions of organisms.
Bil.—Billions of organisms.
Treatment Plan—10 mg *per os* twice a day for 5 days, then 10 mg *per os* once a day for 5 days.

infections produced in mice by the intravenous injections of varying numbers of organisms. The technic of producing such infections and the time and method of treatment have been described previously by us.[3]

It is to be noted that in each experiment, the untreated control mice were dead by the sixth day, and at the end of the first week of treatment 73.5% of those mice treated with sulfapyridine were still alive while only 34% of the mice treated with sulfanilamide were surviving. At the end of the second week (4 days after the termination of therapy) 32.6% of the mice treated with sulfapyridine were alive while only 8% of those treated with sulfanilamide were living. The surviving mice are being held for an indefinite period, to determine their eventual fate.

It seems from these results, that the chemotherapeutic effect of sulfapyridine in staphylococcal infections in mice is definite enough to warrant careful clinical trials of the drug in severe staphylococcal infections. We are conducting such trials and to date we have noted dramatic clinical results in 2 patients suffering from severe staphylococcal sepsis.

10298

Sulfonamide Therapy of Experimental Peritonitis Due to *E. coli, B. proteus* and *B. pyocyaneous.*

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From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh, Pa.

While sulfanilamide therapy of urinary tract infections due to *E. coli* is universally recognized as effective, application of this therapy to similar infections in other sites has received scant attention. For this reason, experiments were undertaken to test the efficacy of sulfanilamide* and of 2-(sulfanilamido)-pyridine† (sulfapyridine) in *E. coli* peritonitis of mice and thereby furnish an experimental basis for possible clinical application. At the same time experiments were also set up to test the value of these drugs in peritoneal infections of *B. proteus* and *B. pyocyaneous* in mice.

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* Synthesized and donated to us by the Monsanto Chemical Company, St. Louis, Missouri.

†Synthesized and donated to us by the Maltbie Chemical Company, Newark, New Jersey.

Method: One of the 2 hemolytic strains of *E. coli* employed, and the strain of *B. proteus,* were both isolated from the pus of an acutely inflamed appendix; the second strain of *E. coli* came from a case of urinary tract infection; while the strain of *B. pyocyaneous* was recovered from a second appendiceal abscess.

Mice were inoculated intraäbdominally with 0.5 cc of the various 6-hour cultures suitably diluted and containing 5% mucin. The animals in the *E. coli* experiment were infected with different cul-

TABLE I.
Curative Action of Sulfanilamide and Its Pyridine Derivative in Peritonitis of Mice Caused by *E. coli*, *B. proteus*, and *B. pyocyaneous.*

				Treatment		No. deaths daily						
Infecting Organism	No. mice	Culture dilution	Lethal doses	20 mg q.d. orally	No. days treated	1	2	3	4	5	6	Alive after 7 days
E. coli	10	10^{-4}	1-10	—	—	7	1	—	—	—	—	2
	10	10^{-4}	1-10	SA	5	—	—	—	1	—	—	9
E. coli	10	10^{-4}	10	—	—	9	1	—	—	—	—	0
	10	10^{-4}	10	SA	2	—	1	—	—	—	—	9
E. coli	10	10^{-3}	1-10	—	—	7	1	—	—	—	—	2
	10	10^{-3}	1-10	P	2	—	2	—	—	—	—	8
E. coli	10	10^{-3}	100	—	—	10	—	—	—	—	—	0
	10	10^{-3}	100	SA	2	2	—	—	—	—	—	8
E. coli	10	10^{-2}	1000	—	—	10	—	—	—	—	—	0
	10	10^{-2}	1000	SA	1	9	1	—	—	—	—	0
B. proteus	10	10^{-2}	10	—	—	10	—	—	—	—	—	0
	10	10^{-2}	10	SA	2	—	1	—	—	—	1	8
B. proteus	10	10^{-2}	10	—	—	8	1	—	—	—	—	1
	10	10^{-2}	10	P	2	1	—	—	—	—	—	9
B. pyocyaneous	10	10^{-1}	1-10	—	—	6	1	—	1	—	—	2
	10	10^{-1}	1-10	SA	2	—	3	—	—	—	—	7
B. pyocyaneous	10	10^{-1}	1	—	—	5	—	—	—	—	—	5
	10	10^{-1}	1	P	—	4	—	—	—	—	—	6
B. pyocyaneous	10	10^{-1}	1	—	—	4	—	—	—	—	—	6
	10	10^{-1}	1	SA	1	—	2	—	—	—	—	8
	10	10^{-1}	1	P	1	—	1	—	—	—	—	9
B. pyocyaneous	10	10^{-0}	10	—	—	10	—	—	—	—	—	0
	10	10^{-0}	10	SA	1	10	—	—	—	—	—	0
	10	10^{-0}	10	P	1	9	—	—	—	—	—	1
B. pyocyaneous plus *E. coli*	10	10^{-3}	10	—	—	10	—	—	—	—	—	0
	10	10^{-3}	10	SA	1	10	—	—	—	—	—	0

SA—sulfanilamide.
P—2-(sulfanilamido)-pyridine.
q.d.—once daily.

ture dilutions, equivalent to 1 to 10, 100, and 1000 lethal doses. Those of the *B. proteus* experiment were given 10 lethal doses, while the mice infected with *B. pyocyaneous* were given less than 1 lethal dose in 2 series and 10 lethal doses in 2 others. In a final experiment, equal parts of 6-hour cultures of *E. coli* and of *B. pyocyaneous* were mixed, diluted to 10^{-3}, admixed with an equal volume of 10% mucin and administered as in the other experiments. This inoculum was equivalent to about 10 lethal doses. All titrations were performed previous to and also simultaneous with the experiments proper.

The treatments, consisting of 20 mg of drug suspended in 0.2 cc of 15% gum acacia, were given by mouth 2 hours after infection and then once daily.

Results: The results listed in Table I show that sulfanilamide was effective against 100 lethal doses, but not against 1000 lethal doses of *E. coli.* Similarly, this drug was effective against 10 lethal doses of *B. proteus* but was ineffective against less than 10 lethal doses of *B. pyocyaneous.* The pyridine derivative of sulfanilamide also proved effective against *E. coli,* and *B. proteus* peritonitis but ineffective against that of *B. pyocyaneous.* Sulfanilamide was ineffective against the mixed infection, although the size of the inoculum of each organism, considered separately, was well within the therapeutic range of this drug.

Summary. Sulfanilamide and 2-(sulfanilamido)-pyridine are both effective against peritonitis of mice caused by *E. coli* and *B. proteus,* but are ineffective against peritonitis of mice caused by *B. pyocyaneous.*

Sulfanilamide may cure mice infected intraäbdominally with 100 lethal doses of *E. coli,* or 10 of *B. proteus;* whereas it is ineffective against 1000 lethal doses of *E. coli;* less than 10 of *B. pyocyaneous;* or against 10 lethal doses of a mixture of *E. coli* and *B. pyocyaneous.*

10299

Chemotherapeutic Evaluation of Sulfanilamide and 2-(Sulfanilamido)-Pyridine in Type II Pneumococcal Infections in Mice and Rats.

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The single report by Whitby[1] of the superiority of 2-(sulfanilamido)-pyridine (sulfapyridine) to sulfanilamide in treating pneumococcal infections of the magnitude of 10,000 fatal doses in mice still lacks confirmation. Since Whitby's results were expressed by a figure representing the average survival time for a 7-day period, there is no indication of the actual number of survivors, except in experiments in which no deaths occurred.

In the following experiments, the 2 drugs were assayed against Type II pneumococcal septicemia in mice and also against Type II pneumococcal meningitis in rats; and the results expressed in terms of actual survivals over a considerably longer period of observation.

In the mouse experiments reported below, all animals were inoculated subcutaneously (for reasons previously mentioned[2, 3]) with between 10 and 100 fatal doses of pneumococci and treated, after infection was established, as shown in Table I. It is evident from the 21-day survival values that 2-(sulfanilamido)-pyridine is somewhat more efficacious than sulfanilamide.

TABLE I.
Therapeutic Efficacy of 2-(Sulfanilamido)-Pyridine and Sulfanilamide Against Type II Pneumococcal Infection of Mice.

	No. of deaths (of 20 mice) daily during 21 days									
Treatment	1	2	3	4	5	6	7	8	9-15	% Survival
None	4	11	5							0
Sulfanilamide*		1	3	5		4	1	1		25
2-(Sulfanilamido)-pyridine†	1			2	3	2	2	1		45

Infection: 0.5 cc of an 10^{-6} broth dilution of an 18-hour Type II (X 2B) broth culture subcutaneously. (Between 10 and 100 fatal doses.)

Treatment: Control group: None. Treated groups: 20 mg of drug suspended in 0.2 cc of 15% gum acacia orally 3, 23, 47 and 72 hours after infection.

*Synthesized and donated to us by the Monsanto Chemical Co., St. Louis, Mo.

†Kindly supplied by Merck and Co., Rahway, N. J.

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[2] Cooper, F. B., Gross, P., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 148.

[3] Cooper, F. B., Gross, P., and Lewis, M., *Am. J. M. Sc.*, 1938, **196**, 343.

Previous studies[3, 4] indicate that a higher percentage of survivals, but a less accurate evaluation of the drugs would doubtless have resulted from a more prolonged course of treatment.

A more critical evaluation of the efficacy of the drugs was obtained by treating rats suffering from well-developed Type II pneumococcal meningitis.[5, 6, 7] The results given in Table II also point to a slight superiority of the new drug over sulfanilamide.

TABLE II.
Therapeutic Efficacy of 2-(Sulfanilamido)-Pyridine and Sulfanilamide Against Type II Pneumococcal Meningitis of Rats.

Treatment	No. of Rats	No. of deaths daily during 21 days											% Survival
		1	2	3	4	5	6	7	8	9	10	11-15	
None	16	7	7	1	1								0
Sulfanilamide	32	2	3	4	3			2				2	50
2-(Sulfanilamido)-pyridine	30	2	2		3			1	1	1	1	2	57

Infection: 0.1 cc of a 10^{-4} broth dilution of an 18-hour Type II (X 2B) broth culture intracranially. (Less than 100 fatal doses.)

Treatment: Control group: None. Treated groups: 100 mg of drug suspended in 0.5 cc of 15% gum acacia orally 6, 13 and 23 hours after infection, then once daily for 8 additional days.

Conclusions. The above experiments, more rigorous than those previously reported[1] because treatment was purposely withheld until infection was established and then conducted with equal doses of the 2 drugs, show 2-(sulfanilamido)-pyridine slightly superior to sulfanilamide in combatting Type II pneumococcal infections of less than 100 fatal doses in mice and rats.* These conclusions are based on survival values only.

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[5] Cooper, F. B., Gross, P., and Lewis, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 835.

[6] Gross, P., Cooper, F. B., and Lewis, M., *Am. J. M. Sc.*, in press.

[7] Gross, P., Cooper, F. B., and Lewis, M., *Am. J. M. Sc.*, in press.

* More recent work, to be published, has shown sulfanilamide superior to 2-(sulfanilamido)-pyridine in combatting experimental Type III pneumococcal pneumonia and meningitis of rats.

10300

Fibrinolytic Properties of Filtrates of *Clostridium histolyticum* in Normal Saline and Dog Serum.

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Within the past few years, considerable study has been devoted to filtrates of *Clostridium histolyticum* grown on various media. This organism, when cultured anaerobically on a protein-rich medium, yields a filtrate displaying marked proteolytic and deaminizing effects[1, 2] upon complex protein substances such as fibrin, casein, coagulated egg albumin and tumor tissue.[3] Studies made on this and other proteinases suggest that they do not have any particular specificity for any single protein complex present in the substrate. According to Diehl[4] the enzyme or enzyme groups constituting the proteinase are formed to correspond to different amino acids in the medium and will attack these whether combined or free. The activity of the proteinase of *Cl. histolyticum* depends on pH. However, various optima ranging from pH 7.0[2] to pH 8.0-9.0[5] have been reported. Some workers have found more acid optimum values.[6] Filtrates of *Cl. histolyticum* are also believed to show increased proteolytic activity in the presence of sulphydryl compounds and various metallic ions.[1, 2, 7]

Connell and his associates[3] have used parenteral injections of filtrates of the organism grown on specific tumor tissue in the hope that such material might possess sufficient enzymatic specificity to attack tumor tissue *in vivo*. Their results have been neither thoroughly confirmed nor refuted.[8, 9, 10] The original purpose of the short series of experiments to follow was to determine the fibrinolytic

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* Aided by the A. D. Nast Fund for Cardiac Research.

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[2] Weil, L., and Kochalaty, W., *Biochem. J.*, 1937, **31**, 1255.

[3] Connell, H. C., *Canad. Med. Assn. J.*, 1935, **33**, 364.

[4] Diehl, H. S., *J. Infect. Dis.*, 1919, **23**, 347.

[5] Walburn, L. E., and Reymann, G. C., *J. Path. and Bact.*, 1934, **39**, 669.

[6] Blanc, J., and Pozerski, E., *Comp. rend. Soc. Biol.*, 1920, **83**, 1369.

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[9] Rusch, H. P., and Preston, W. S., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 581.

[10] Moppett, W., and Goldsworthy, N. E., *Med. J. Australia*, 1936, **23**, 754.

activity of filtrates of *Cl. histolyticum* grown on various media with particular reference to their possible use in clinical conditions associated with intravascular fibrin deposition and clotting, for example subacute bacterial endocarditis.

The culture of *Cl. histolyticum* used by Weil and Kochalaty[2] (American Type Culture Collection No. 4972) was obtained for these experiments. The organism was cultured anaerobically on various media for 4 days at 37°C, after which the material was passed through a Zeitz filter. The filtrate was added in varying amounts to normal saline (pH 6.7) on the one hand, and dog serum (pH 7.2) on the other to produce 10 cc of mixture in which the filtrate dilution was 2.5, 5.0, 10, 20, and 30% respectively. In each test tube a block of sterile dog fibrin weighing approximately 0.5 g was immersed. The test tubes were shaken several times daily and kept at a temperature of 37°C. All procedures were performed with sterile technic. Preliminary experiments showed that the addition of cysteine monohydrochloride as activator was without appreciable effect. The index of fibrinolytic activity (F) was arbitrarily determined by the formula,

$$F = \frac{1}{T \times C}$$

T = time in days for complete fibrin liquefaction and C = percentage concentration of filtrate producing liquefaction.

TABLE I.

Culture Medium	Normal Saline			Dog Serum		
	C	T	F	C	T	F
Neopeptone broth	2.5	1	.40	20	3	.017
Horse meat broth	2.5	1	.40	5	7	.029
Clotted dog blood*	2.5	1	.40	5	5	.040
Dog serum	2.5	1	.40	20	3	.017
Particulate fibrin masses in normal saline	2.5	3	.13	No effect		

F = fibrinolytic index.
T = time in days for complete liquefaction of fibrin.
C = percentage concentration of filtrate producing liquefaction.
*Liquefied by growth.

From Table I it appears that in several rich protein media, the *Cl. histolyticum* produces filtrates with high fibrinolytic potency. It is also evident that this potency is tremendously decreased in the presence of dog serum. When grown on a simple medium of fibrin in saline, a filtrate of low potency was produced. Controls containing no filtrate did not produce fibrin liquefaction.

The antienzymatic power of serum has long been recognized and its effect on various enzymes studied, for example, trypsin,[4, 11] leucoprotease,[12] lipase, etc. Blanc and Pozerski[6] found decreased power of gelatin liquefaction of *Cl. histolyticum* filtrates in the presence of raw serum albumin. Other enzymes have been studied more extensively in this respect.[13] Furthermore, the result of repeated injection of such materials into animals is to increase the antienzymatic effect of the serum[14, 15] and to produce antibodies.[16] The exact nature of the substance involved in the antienzymatic mechanism it not yet completely understood. It seems reasonable to assume from our experience and that of others with *in vitro* experiments, that such fibrinolytic filtrates would not be effective *in vivo*.

Summary. 1. Filtrates of *Cl. histolyticum* grown on various protein-rich media possess a high fibrinolytic potency, whether sulphydryl activator be present or not. 2. This fibrinolytic potency is greatly depressed in the presence of dog serum. 3. It is probable that the use of filtrates of *Cl. histolyticum* as a fibrinolytic agent in dissolving fibrin deposits *in vivo* would not be feasible due to the antienzymatic properties of blood serum.

I am grateful to Dr. L. N. Katz, to Dr. K. Howell, and to Dr. I. Kaplan for helpful suggestions and advice.

10301

Heparin and the Blood Coagulation Mechanism.*

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The efficacy of heparin as an anticoagulant was first observed by Howell and Holt.[1] Later Howell[2] demonstrated the presence of heparin in the blood and described a method for its purification.

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[14] Achalme, P., *Ann. de l'Inst. Pasteur*, 1901, **15**, 737.

[15] Bergmann, von, und Bamberg, *Berl. klin. Wochenschr.*, 1908, **45**, 1396.

[16] Connell, H. C., *Bull. Hendry-Connell Research Foundation*, 1938, **3**, 3.

* Assisted by a grant from the Christine Breon Fund.

[1] Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918, **47**, 328.

[2] Howell, W. H., *Am. J. Physiol.*, 1924, **71**, 553.

Quick[3, 4] demonstrated that, although heparin itself is not an antithrombin, it does "appear to react with a constituent present in plasma to form a true antithrombin." He also presented data to show that thromboplastin does not neutralize heparin. Removal of the electrolytes (and perhaps of a "diffusible organic substance"[5]) from plasma renders heparin inactive. Eagle[6] stated that "it is debatable whether heparin is of *physiologic*† significance in preventing intravascular coagulation," but concluded in his summary that "heparin apparently prevents coagulation both by retarding the formation of thrombin and by acting as an antithrombin." Charles and his coworkers[7, 8] prepared a potent product of heparin which could be administered intravenously. Murray and Best and their coworkers[9, 10] used this material (70 units per mg) on animals and human subjects and demonstrated that it greatly reduced or prevented the tendency to vascular occlusion in traumatized veins. Crafoord[11] used heparin (prepared by Jorpes) post-operatively in human subjects and noted that it produced prolongation of the coagulation time of the blood without any toxic after-effects.

Our experiments consisted of determinations of the effects of heparin‡ on the coagulation time of whole blood and plasma *in vitro* and *in vivo* in normal and in diseased subjects.

All experiments were performed in a water-bath maintained at a temperature of 37°C. The Lee and White method for the coagulation time of whole blood was used. The methods of Quick, with slight modifications, were employed for the coagulation time of recalcified plasma, and of recalcified plasma to which an addition of thromboplastin was made.[12, 13, 14] A 5% emulsion of fresh rabbit

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[3] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

[4] Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 391.

[5] Larson, C. E., and Greenberg, D. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 305.

[6] Eagle, H., *Medicine*, 1937, **16**, 95.

† Author's italics.

[7] Charles, A. F., and Scott, D. A., *J. Biol. Chem.*, 1933, **102**, 425, 437.

[8] Charles, A. F., and Scott, D. A., *Trans. R. S. Can.*, 1934, **28**, Sec. V, 55.

[9] Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H., *Can. Med. Assn. J.*, 1936, **35**, 621.

[10] Best, C. H., Cowan, C., and MacLean, D. L., *Science*, 1937, **85**, 338.

[11] Crafoord, C., *Acta Chir. Scand.*, 1937, **79**, 407.

‡ Heparin obtained from the Connaught Laboratories. A sterile solution for intravenous use, 1000 units per cc. One unit of this product will keep 1 cc of cat blood free of clots for 24 hours in cold-storage.

[12] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

[13] Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

[14] Aggeler, P. M., and Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 11.

brain provided the source of thromboplastin. The results of the experiments undertaken are tabulated; in each instance the figures shown were characteristic of all experiments of the series.

EXPERIMENT I.

Effect of Varying Concentrations of Heparin on the Coagulation Time of Normal Whole Blood and Plasma *in vitro*. (Results in one of 5 experiments.)

	Coagulation Time		
Concentration of heparin-units per cc	Whole blood	Recalcified plasma, saline control	Recalcified plasma with added thromboplastin
1.5	no clot in 360′	75′0″	720″
1.0	46′	10′0″	42″
0.5	35′	8′15″	35″
0.25	25′	5′45″	27″
0.1	9′	3′10″	22″
0.05	5′	2′45″	22″
Control	5′	2′10″	22″

EXPERIMENT II.

Effect on Coagulation Time of Whole Blood and Plasma of a Single 3 cc Dose (3000 Units) of Heparin Administered Intravenously to a Normal Subject. (Results in one of 5 experiments.)

	Coagulation Time		
Interval	Whole blood	Recalcified plasma, saline control	Recalcified plasma with added thromboplastin
Before heparin (control)	5′	2′10″	22″
5 min. after heparin	53′	5′45″	39″
1 hr " "	25′	4′10″	29″
2 " " "	12′	2′15″	24″
3 " " "	6′	2′10″	22″
4 " " "	5′	2′12″	22″

EXPERIMENT III.

Effect on Coagulation Time of Whole Blood and Plasma of a Single 3 cc Dose (3000 Units) of Heparin Administered Intravenously to a Hemophiliac and a Patient with Icterus (Index 125).

	Coagulation Time			
	Whole blood		Recalcified plasma with added thromboplastin	
Interval	Hemophilia	Icterus	Hemophilia	Icterus
Before heparin (control)	45′	5′	21″	29″
5 min after heparin	240′	42′	39″	45″
1 hr " "	135′	23′	26″	34″
2 " " "	90′	12′	21″	29″
3 " " "	48′	6′	20″	28″
4 " " "	44′	6′	21″	29″
Normal patient control	6′	5′	22″	22″

Comparing Experiments II and III reveals that the coagulation time of the whole blood of the hemophiliac differs markedly from the normal, while that of the recalcified plasma remains similar. In icterus, it is the coagulation time of the recalcified plasma which differs from the normal.

EXPERIMENT IV.
Effect on Coagulation Time of Whole Blood of Single Graded Doses of Heparin Administered Intravenously to Normal Subjects. (Results in one of 5 experiments.)

Interval	Coagulation time of whole blood after 1 cc	2 cc	3 cc	4 cc	6 cc
Before heparin (control)	5′	4′30″	5′	7′30″	5′
5 min after heparin	17′	27′	53′	90′	100′
30 ” ” ”	13′	22′			
1 hr ” ”	11′	15′	25′	53′	60′
1½ ” ” ”	6′30″	11′			
2 ” ” ”	5′	8′	12′	28′	37′
3 ” ” ”		5′	6′	15′	16′
4 ” ” ”			5′	8′	9′
5 ” ” ”				7′	5′

A relatively large dose of heparin given intramuscularly is practically without effect. A 5 cc intramuscular dose given simultaneously with a 3 cc intravenous dose did not prolong the coagulation time of the whole blood significantly insofar as intensity or duration of the effect is concerned. A continuous intravenous drip of salt solution delivering 1000 units of heparin per hour was without effect. If an initial intravenous dose of 3 cc of heparin is given and followed by a continuous intravenous dose of 1 cc (1000 units) per hour, the coagulation time of the whole blood may be kept at 30 minutes for periods of 8 hours or more.

EXPERIMENT V.
Effect on Coagulation Time of Whole Blood and Plasma of Repeated Single Doses of Heparin. (One of 4 experiments.)

	Coagulation Time		
Interval	Whole blood	Recalcified plasma, saline control	Recalcified plasma with added thromboplastin
Before heparin (control)	7′	2′15″	22″
3 cc heparin intravenously			
5 min. after heparin	48′	4′30″	33″
1 hr ” ”	28′	2′33″	28″
2 ” ” ”	14′	2′30″	26″
2 cc heparin intravenously			
5 min after heparin	50′	3′25″	30″
1 hr ” ”	26′	2′45″	27″
2 ” ” ”	15′	2′40″	25″
2 cc heparin intravenously			
5 min after heparin	54′	3′45″	33″
1 hr ” ”	31′	3′02″	27″
2 ” ” ”	20′	2′55″	25″
3 ” ” ”	10′	2′30″	23″
4 ” ” ”	8′	2′20″	22″

In 2 persons suffering from coronary artery thrombosis, 3 cc doses of heparin repeated at 3 hourly intervals were given for periods of 5 and 7 days. The coagulation time was kept prolonged throughout this period.

During the course of these experiments some evidence was obtained to indicate that the heparin injected was probably destroyed in the body and not voided in the urine. The bleeding time (Duke method), capillary fragility (Daldorf method) and clot retraction after heparin are normal. Leucocytes and bone marrow pulp left in contact with heparin for periods greater than one hour tend to agglutinate and later disintegrate. There is a tendency for hematomata to form at the site of venipuncture after heparin unless the wound is carefully tamponed.

Summary. Heparin added to whole blood *in vitro* or administered by vein prolonged the coagulation time of whole blood and plasma. The coagulation time was prolonged and maintained by a single large dose of heparin followed by a smaller continuous dose. The mechanism appeared to be independent of the presence and type of disease. Heparin produces agglutination and disintegration of leucocytes and bone marrow pulp *in vitro*.

10302

Serological Studies on Mastitis.

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One of the great difficulties encountered in studies on the streptococcal type of mastitis is the diagnosis of the chronic form of the disease. Both the chemical and bacteriological methods have their proponents, but as yet, no one test or series of tests can be considered as a final diagnostic measure. For this reason it would seem that any valuable means of diagnosis must bring in methods differing greatly from those already recommended.

Despite the fine work done on certain animal diseases by applying serological methods this particular field seems to have been practically neglected by those interested in mastitis. This appears particularly strange in view of the fact that as early as 1904 Stahli[1] was able to demonstrate agglutinating antibodies in the serum of a mastitic cow. He also found that these antibodies were absent from the sera of uninfected cattle. The major criticism of this work would seem to be that it was limited to one cow and to a single strain of streptococcus.

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[1] Stahli, A. T., *Arch. f. wessensch. u. prakt. Tierheilk.*, 1904, **30**, 374.

Recently Stableforth[2] made an extensive survey on the types and sub-types of *Streptococcus mastitidis (Streptococcus agalactiæ)* in the herds of Great Britain. He found that usually the strains present in a given herd were alike. The author also believed that serological examination may be used as a diagnostic means in mastitis. His method involved isolation of the organism from the suspected cow followed by agglutination-tests with each of his 5 types of antisera. Stableforth states, "The experience gained in the work here reported has shown that serological methods offer a ready and reliable means of recognizing *Str. agalactiæ* and these have, during the past 3 years, gradually replaced biochemical methods in the control of *Str. agalactiæ* infections."

The work of Lancefield[3] has entirely revolutionized the serological study of streptococci, and thus it appeared desirable to apply her methods in the study of mastitis. If the invasion of *Streptococcus mastitidis* into the udder of the cow is followed by the appearance of antibodies in the serum of that cow, and if these antibodies are group-specific then we would have a very valuable and simple means in the diagnosis of the streptococcal type of mastitis. Extracts of any typical Group B organism would then react with positive serum from any cow. If, however, the antibodies produced are type-specific then precipitative and agglutinative procedures will become much more complicated. The testing of type-antisera would probably necessitate the use of a number of strains of *Streptococcus mastitidis.* This number, however, from the work of Stableforth[2] does not appear to be prohibitory.

Two cultures of *Streptococcus mastitidis* were used in this study. One of these was a typical Group B organism isolated from human feces[4] while the other was isolated from an acute case of mastitis in one of the herds under observation. This latter organism was not subjected to the Lancefield test but did correspond to the physiological reactions described by Sherman[5] for *Streptococcus mastitidis.* It would not seem likely that these 2 organisms would belong to the same serological type under Group B.

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[2] Stableforth, A. W., *J. Path. and Bact.*, 1938, **46**, 21.

[3] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

[4] Smith, F. R., and Sherman, J. M., *J. Infect. Dis.*, 1938, **62**, 186.

[5] Sherman, J. M., *Bact. Rev.*, 1937, **1**, 1.

Sera for these studies were obtained by bleeding from the jugular vein. Precipitin-tests were made according to the method recommended by Lancefield.[3] The agglutinative procedure was essentially that described by Griffith.[6]

Two series of blood samples were taken from Herd I. An interval of 2 months elapsed between the first and second bleedings. This herd was known to contain mastitic cows and it was from one of the cows in this group that the bovine strain of *Streptococcus mastitidis* was isolated. Only one series of blood samples was secured from Herd II. Little was known about the history of this herd except that it had no connection with Herd I.

All Lancefield tests on the Group B streptococcus isolated from human feces were negative. Thus it would seem that group-antibodies are not produced in the sera of cows infected by *Streptococcus mastitidis*.

Serological reactions on the strain isolated from an acute case of mastitis were interesting. These results have been summarized in Table I. There would appear to be no doubt that type-specific immune bodies are present in the sera of certain cows. Since the complete history of the herd was lacking it would be impossible to correlate the presence of these immune bodies with the incidence of infection in the herd. Routine laboratory analysis of Herd I before the serological studies were begun would indicate that the agglutinative method would discriminate against many cows giving apparently normal milk. This is to be expected since the antibody titer of the blood should remain relatively high for some time after symptoms of infection have ceased.

Due to the large number of positive agglutinative reactions with Herd I it would certainly appear that the infection of this herd was

TABLE I.

Serum reactions with *S. mastitidis* isolated from an acute case of mastitis in Herd I		Serum from individual cows in:		
		Herd I		Herd II
		4/6/38	6/16/38	6/16/38
Agglutination Lancefield	+ —	54	36	0
Agglutination Lancefield	— +	0	2	0
Agglutination Lancefield	+ +	30	15	0
Agglutination Lancefield	— —	33	64	17

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[6] Griffith, F., *J. Hyg.*, 1934, **34**, 542.

due to one specific type of Group B. This conclusion coincides well with the observation of Stableforth.[2]

The total lack of reaction in the case of Herd II is very interesting, and does indicate that there is some correlation between the occurrence of a given type of *Streptococcus mastitidis* in the herd and the presence of antibodies in the sera of that herd.

It is not the purpose of this work to advocate the routine use of serological methods in the diagnosis of mastitis. Considerable work would be necessary before one would be justified in drawing conclusions as to the correlation between mastitis and the presence of agglutinating antibodies. Such data would necessitate a survey such as that conducted by Stableforth in order that we may attain some knowledge of the number of types of *Streptococcus mastitidis* encountered in the United States. Furthermore complete studies should be made on herds in order that we may draw conclusions regarding the correlation between mastitis as shown by our more common tests and the agglutinative response. Until such data have been collected the serological reactions of cow serum can only be of theoretical interest.

Conclusions. Cow sera may contain streptococcal antibodies which appear to be type-specific. There is evidence of a correlation between the presence of *Streptococcus mastitidis* in the herd and the occurrence of agglutinating antibodies in the serum of the individual cows. It appears probable that infection in an individual herd may be due to a specific type under Group B.

10303 P

Sputum Studies in Lobar Pneumonia. Extra-Cellular Encapsulated Pneumococci.

ARTHUR W. FRISCH. (Introduced by W. O. Nelson.)

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In a previous report concerning rusty sputum from cases of lobar pneumonia, 3 types of phagocytosis and the effect of serum therapy were described.[1] The present communication deals with the number of extracellular capsulated pneumococci in rusty sputum as an index of the severity of the pneumonia. No attempt will be made at present to relate the counts to therapy.

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[1] Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 473.

Seventy-eight roentgenographically proved cases of pneumonia with the following type distribution have been studied: 20 cases of type I, 30 type II, 3 type III, 2 type V, 1 type VI, 11 type VII, 3 type VIII, 1 type IX, 3 type XII, 1 type XVI, and 2 mixed. Thin smears were made directly from representative rusty portions of the sputum and treated with Wright's blood stain for 3 to 7 minutes. Without the addition of buffer the stain was flooded off with water and the slides were air dried. The capsules of the pneumococci usually did not stain but were readily visualized as clear or occasionally as pink zones around the dark blue diplococci. The mucin stained pale pink and the leukocytes were differentiated as on a blood smear. At first, samples of rusty sputum were examined at 4- to 6-hour intervals throughout the acute stages of the disease. However, specimens taken every 12 hours were found to be equally satisfactory and most of the cases were followed in this manner.

During the study it was found that rusty sputum, relatively free of organisms other than pneumococci, gave the most consistent results. Frankly purulent specimens contained large numbers of capsulated pneumococci which had no relation to the clinical course or outcome of the pneumonia. Mucoid sputa early in the disease are now under investigation.

The number of extracellular capsulated diplococci per oil immersion field varied considerably on different parts of the same slide depending upon the thickness of the smear. Approximately 50 representative fields were counted at first and the average number per field was estimated considering each pair of cocci as one organism. Counts of 2 slides made from different portions of each specimen of sputum and determined separately by 2 individuals usually agreed within 10%. At present, fairly accurate checks are obtained by counting only 10 fields. Because one is dealing with individual samples of sputum which probably originate from various portions of the infected lung, one might expect that the number of free pneumococci in specimens obtained at frequent intervals would fluctuate sufficiently to invalidate the results. Actually the counts from sputum to sputum in the same patient were surprisingly constant and the increase or decrease in the number of pneumococci per oil immersion field was progressive rather than sudden.

In Table I under 4 subdivisions (A, B, C, D) are listed the highest number of extracellular capsulated pneumococci per oil immersion field obtained in the sputum of the patients at any time during the

TABLE I.

	Group A 0 to 10	Group B 11 to 30	Group C 31 to 65	Group D Over 65
No. of cases	31	22	10	15
Positive blood culture	2	9	7	11
Leukopenia (less than 10,000)	1	3	5	7
More than one lobe involved	9	5	4	11
Number of deaths	1	2	2	13

acute stages of the disease. These counts were compared with data* of known prognostic significance from the same cases.

From the table it may be noted that the incidence of unfavorable prognostic signs such as positive blood culture and leukopenia was roughly proportional to the number of extracellular capsulated pneumococci per oil immersion field. The high mortality in those cases with more than 65 free pneumococci per field (Group D) coincided with the greater incidence of positive blood cultures and leukopenias. The single death in Group A was, in the author's opinion, due largely to asphyxia from thick purulent sputum and occurred in a case of type II pneumonia with a negative blood culture and a leukocytosis. The sputum at the time of death showed an occasional type II pneumococcus and myriads of influenza bacilli. The two deaths in Group B and one in Group C occurred in patients with type III infections and were associated with leukopenia and positive blood cultures. In these and other fatal cases of type III pneumonia the count of extracellular pneumococci in the sputum and in the lung at autopsy has been consistently low. The other death in Group C occurred during aspiration of an interlobar empyema in a patient convalescing from a severe type II pneumonia. The deaths in group D were, for the most part, fulminating pneumonias in patients who either came to the hospital moribund or in whom the therapy seemed to have no effect. Of the 2 type I cases who survived in Group D, one showed a high sputum count in only one specimen and the second recovered after a difficult infection which was complicated by empyema, gluteal abscess, and a panophthalmitis which necessitated the removal of one eye. Type I pneumococci were recovered from all the lesions.

If it can be shown that the extracellular capsulated pneumococci in rusty sputum reflects the number present in those areas of the lung where the pneumonic process is advancing, then the method and the results presented in this preliminary report are encouraging as a means of evaluating the severity of a given case of pneumonia on admission of the patient to the hospital and during the course of the disease. Further study of a large series of cases will be necessary to establish this point.

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* Data obtained through the courtesy of Doctors A. E. Price and G. B. Myers in charge of the Commonwealth Fund pneumonia study at Receiving Hospital.

10304

Serial Production of Phage from Intracellular Phage Precursor.*

A. P. KRUEGER AND E. J. SCRIBNER.

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Recently experimental evidence was advanced indicating that phage production occurs according to the reaction: inactive phage precursor + phage → phage.[1] The particular point of interest in this concept is the dissociation of phage production from what was previously held to be an essential conditioning factor, namely, bacterial growth. Furthermore, there exist authentic models for such a reaction in the autocatalytic transformation of inactive enzyme precursors into the active enzymes when the precursors are brought in contact with small amounts of active enzyme.

To summarize the experimental data it was found by Krueger and Baldwin[1] that under certain conditions cell-free ultrafiltrates of staphylococci added to solutions of bacteriophage resulted in 100% increases in phage activity. In studying the effect of salts on the phage-bacterium reaction it was found that the presence of sodium chloride[2] or sodium sulphate[3] during the phage-bacterium reaction produced a prelytic plateau in the bacterial growth curve for as much as 0.8 hour during which time phage production continued at a normal rate despite the absence of cellular reproduction. Later[4] experiments were reported in which phage production was dissociated from bacterial growth by means of changes in temperature and pH of the medium. Unfortunately these data do not offer conclusive proof that the phage precursor-phage reaction is the normal mode of phage production largely because the yields of pre-

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*This work was supported by grants-in-aid from the Research Corporation, the American Medical Association, and several interested friends.

[1] Krueger, A. P., and Baldwin, D. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 393.

[2] Krueger, A. P., and Scribner, E. J., *J. Gen. Physiol.*, 1937, **21**, 1.

[3] Krueger, A. P., and Strietmann, W. L., *J. Gen. Physiol.*, 1938.

[4] Krueger, A. P., and Fong, J., *J. Gen. Physiol.*, 1937, **21**, 137.

cursor in ultrafiltrates are small and irregular and also because the methods used to detect Δ [bacteria] in the pH-temperature experiments were not sufficiently sensitive. Continued attempts to improve the methods for extracting precursor were unsuccessful and for the past year we have been working with a method which demonstrates the phage precursor within the bacterium. The present paper deals with the serial production of phage in separate aliquots of precursor-containing bacteria.

In the general procedure for demonstrating phage precursor[5] it is necessary to prepare suspensions of activated staphylococci by growing the organisms in a highly oxygenated medium. The details of this process are described in a paper now in press. After activation the bacteria are separated from the medium, resuspended in Locke's solution and maintained at 5°C for 2 hours in order to rule out the possibility of bacterial growth during subsequent reactions with phage. To demonstrate the precursor 4 ml of activated suspension containing 5×10^8 bacteria per ml is added to 1 ml of phage diluted with Locke's solution to contain 1×10^9 activity units per ml. The mixture is kept for 5 minutes at 5°C and is then titrated for total phage content by the activity method. The end titer is found to be 2×10^9 phage units per ml while that of the control prepared with nonactivated bacteria from a 16-hour agar culture is 2×10^8 units per ml.

The objection may be advanced that the increase in activity is a spurious one and involves some untoward effect of the activated cells on the titration system. This is apparently upheld by the fact that plaque counts show no increase in phage content of the activated bacteria-phage mixture. However, control experiments (Table I) have been run, adding the number of activated bacteria which would occur in samples of activated bacteria-phage mixtures when diluted for titration to standard titration mixtures. As shown in Table I these small amounts of activated bacteria do not influence the titration values. The failure of the plaque count to increase is explicable on the basis that no matter how much intracellular phage is formed per organism, the count will remain the same because each phage-containing bacterium becomes the locus of one phage plaque regardless of the amount contained in the cell.

The ideal test for phage precursor would be the demonstration that a small amount of phage added to successive aliquots of precursor would result in the continuous transformation of precursor in each aliquot into more phage. Since it is exceptional to obtain

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[5] Krueger, A. P., and Mundell, J., *Science*, 1938, **88**, 550.

TABLE I.

Outline of Experiment to Test Effect of Activated Bacteria Added to Phage Titration System.

1. 9 ml broth blanks prepared containing three different concentrations of activated bacteria:

    5 x 10^5 activated bacteria/ml
    5 x 10^4 ” ” /”
    5 x 10^3 ” ” /”

2. Sets of each concentration used to dilute standard phage for titration (from 1 x 10^{10} activity units/ml to 1 x 10^7, 1 x 10^6, and 1 x 10^5 activity units/ml).
3. To 4 ml of each dilution of phage 1 ml of normal bacteria containing 12.5 x 10^7 cells/ml was added.
4. Time of lysis was determined.
5. Presence of 5 x 10^3, 5 x 10^4, or 5 x 10^5 activated bacteria/ml in titration system does not affect results.

sufficient concentrations of cell-free precursor to permit this test being done it is necessary to use intracellular precursor for the test. Immediately the difficulty arises that when phage is taken up by activated bacteria and converts the intracellular precursor into more phage practically all of the phage remains in or on the bacterium and consequently is not free to participate in transforming additional precursor into more phage. However, if appropriate conditions are chosen the cells may be lysed by the phage and the newly formed phage liberated free in solution.

Ordinarily there are required for lysis about 100 phage units per bacterium[6] and standard phage contains 1 x 10^{10} activity units per ml. These two facts limit the number of bacteria which can be lysed (without growth) unless a concentrated phage is used. In an average mixture when the concentrations requisite for lysis obtain there are 5 x 10^9 phage units per ml and 5 x 10^7 bacteria per ml. Under these conditions the phage concentration is so high and the number of bacteria so small that the increment of newly formed phage derived from the precursor-containing cells is both too small to detect and too small to permit a serial dilution experiment. This difficulty may be circumvented by carrying out the activation in the presence of $MnCl_2$ for it has been shown[7] that manganese-treated cells have a much lower lytic threshold than untreated bacteria, *i. e.*, they require less phage for the initiation of lysis.

Details regarding the strain of staphylococcus used, the media employed, etc., are given in earlier publications. For the present work 16-hour agar cultures of the staphylococcus were washed twice in Locke's solution and were then activated by growth in a medium through which oxygen was bubbled. The exact procedure for activation as described by Krueger and Scribner[8] was followed except that in this case the medium contained 0.0016M $MnCl_2$. Upon the completion of activation the bacterial suspension was placed in ice mixture and was kept for 2 hours before using.

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[6] Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223.

[7] Krueger, A. P., and West, N. S., *J. Gen. Physiol.*, 1935, **19**, 75.

Two controls were routinely employed in testing for activation:

1. A mixture containing 5×10^8 activated bacteria per ml and 2×10^8 phage units per ml was made in Locke's solution. This suspension was kept at 5°C for 5 minutes and was immediately diluted for titration, using for this purpose 0.0016M Na_2SiO_3 broth in order to prevent any effect of the manganese on the titration system.

2. A duplicate of number 1 except that nonactivated bacteria from the same culture were used and before mixing with phage these organisms were washed in 0.0016M $MnCl_2$ broth.

For transforming intracellular precursor into phage under conditions such that the cells would lyse and set free the newly formed phage, mixtures of the following composition were prepared: 6.3 ml of phage containing 1×10^9 activity units per ml, 2 ml of broth containing 0.008M $MnCl_2$, 1.7 ml of broth and 1 ml of bacterial suspension activated in the presence of $MnCl_2$ and containing 1×10^9 cells per ml. The mixtures were kept on ice for 0.75 hour and were then transferred to the waterbath shaker at 36°C. Lysis began between 0.5 and 0.6 hour and was complete between 0.8 and 0.9 hour after which the lysates were promptly titrated for phage content. As soon as the titer was known the lysate was again diluted to contain 1×10^9 phage units per ml and was used in preparing the next mixture in the series. In this way successive dilutions of the original amount of phage were prepared, adding each time new aliquots of activated bacteria.

It was necessary, of course, to prove that no cellular growth occurred during the interval before lysis took place. For this purpose plate counts were taken at short intervals from control mixtures made without phage and containing an aliquot of the organisms activated in the presence of $MnCl_2$. As an additional rough check all the mixtures were compared at 0.2 hour intervals with turbidity standards but reliance was placed only on the plate counts. In none of the control set did growth occur before lysis had taken place in the corresponding phage-containing mixture.

Results. As shown in Table II it has been possible by starting with a small amount of phage to transform the phage precursor in successive lots of staphylococci into phage under the conditions of

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[8] Krueger, A. P., and Scribner, E. J., in press.

TABLE II.
Serial Production of Phage from Successive Aliquots of Precursor-Containing Bacteria.

	Mixture No. 1	Mixture No. 2	Mixture No. 3	Mixture No. 4	Mixture No. 5	Mixture No. 6
Initial [Phage]	6.3 x 10^8	6.3 x 10^8	6.3 x 10^8	6.3 x 10^8	6.3 x 10^8	6.3 x 10^8
Initial [Bacteria]	1 x 10^8	1 x 10^8	1 x 10^8	1 x 10^8	1 x 10^8	1 x 10^8
	Diluted to ↗	Diluted to ↗	Diluted to ↗	Diluted to ↗	Diluted to ↗	
Final [Phage]	7.9 x 10^9	9.5 x 10^9	6.3 x 10^9	7.6 x 10^9	5.7 x 10^9	6.3 x 10^9
Total [Phage] *i. e.*, Initial [Phage] × Total Dilution	6.3 x 10^9	7.87 x 10^{10}	11.8 x 10^{11}	11.8 x 10^{12}	14.2 x 10^{13}	12.8 x 10^{14}

the experiment. In ordinary suspensions of activated organisms the serial dilution experiment is not possible because of the high lytic threshold, *i. e.,* the ratio phage/bacteria is so great that the ultimate yield of phage derived from precursor-containing cells is not measurable. The presence of $MnCl_2$ lowers the lytic threshold very considerably and makes it feasible to add relatively large numbers of organisms with the result that significant amounts of phage are released from the lysing bacteria. The phage produced in this way can then be diluted and employed to activate the precursor of a new lot of cells. The original phage used has been diluted to at least 1/1,000,000 without any reduction in activity or plaque count. This we interpret as indicating that the activated bacteria contain some sort of a phage precursor which is transformed into active phage by phage itself. An alternate explanation would postulate the phage to be a living autonomous unit, finding appropriate conditions for multiplication in the successive aliquots of activated organisms. However, there is ample evidence that phage is not a living organism but rather is a protein of high molecular weight possessing the general properties of an enzyme.[9, 10] It seems probable that phage production is not dependent upon bacterial reproduction *per se* but upon environmental conditions adequate for the normal synthesis of phage precursor.

10305

Experimental Pancreatico-Gastrostomy. Method for Conserving External Secretions of Pancreas.

E. Cooper Person, Jr., and Frank Glenn. (Introduced by Bruce Webster.)

From the Department of Surgery, the New York Hospital and Cornell Medical College.

In any work on the pancreas, the well known danger of acute pancreatitis and peritonitis has long invested the gland with the reputation of a surgical *"noli me tangere."* The surgical attack has also been delayed because of the organ's relative inaccessibility, complicated functions and intimate relationship with major abdominal structures. Nevertheless, the need for a surgical procedure to pre-

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[9] Northrop, J. H., *J. Gen. Physiol.,* 1938, **21**, 335.

[10] Krueger, A. P., *Physiol. Rev.,* 1936, **16**, 1.

pare a new exit for the external secretions of the pancreas applicable to cases of duct occlusion has long been felt.

The first pancreatic transplant was reported by Biondi who implanted the pancreatic segment into the duodenum. His experiment was unsuccessful; all 6 dogs on which the procedure was attempted died of peritonitis. Stimulated by Desjardins'[1] criticism of Biondi's[2] procedure, Coffey[3] described an original and delicate technic for the successful transplantation of the pancreas into the jejunum. This work was verified, using a simplified technic by Sweet and Simons,[4] and Patrie, Pyle and Vale.[5]

The first successful transplant of the pancreas into the stomach was done by Tripode and Sherwin.[6] They chose this procedure because of the accessibility and size of the organ as well as to eliminate any possible obstruction of the lumen of the bowel. Because this method of blind implantation is not in accordance with modern surgical principles, we felt that a direct visualization of the implanted pancreas constituted an improvement.

The pancreatic transplants were carried out on 25 dogs. The transected end of the pancreas was drawn through an incision in the posterior wall of the stomach by stay sutures visualized through an incision in the anterior wall. The anterior wall incision made possible direct observation of the implanted, transected segment in the lumen of the stomach.

Of the 25 animals subjected to this procedure 9 are still alive. Of 16 only one died of acute pancreatitis due to a technical error. The remaining 15 died as result of further surgical procedures. Autopsy in these cases revealed the transplanted gland to be normal.

In Chart I are presented data of 5 living and 5 expired animals.

All dogs were placed on a standard diet high in vitamins A, B, and C. They remained active and healthy and as shown maintained their weight. At varying intervals following the transplant, analysis of gastric contents, blood sugar and urine were made. Since gastric analyses revealed the presence of pancreatic lipase it must be concluded that pancreatic secretions reached the stomach. The blood sugar varied from 60-90 mg/100 cc and the urine analysis failed to show the presence of sugar. Routine abdominal exploration

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[1] Desjardins, A., *Revue de Chirurgie*, 1907, **1**, 945.

[2] Biondi-Dot, D., *La Clinica Chirurgica*, 1896, **4**, 135.

[3] Coffey, R. C., *Ann. Surg.*, 1909, **50**, 1238.

[4] Sweet, J. E., and Simons, I. H., *Ann. Surg.*, 1915, —, 309.

[5] Patrie, H. H., Pyle, L. A., Vale, C. F., *Surg., Gyn., Obs.*, April, 1917, 479.

[6] Tripode, A. M., and Sherwin, C. F., *Arch. Surg.*, 1934, **28**, 345.

CHART I.

Studies upon Dogs in Which a Portion of the Pancreas Was Transplanted into the Posterior Wall of the Stomach and the Remainder of the Pancreas Resected. Gastric lipase positive, blood sugar normal, urine negative, liver micros. fatty infiltration negative.)

Dog No.	Initial wt (lb)	Days after procedure	Present wt or at death	General health	Cause of death	Quantitative liver lipid %	Re-exploration post-mortem	
							Gross	Microscopic
251	20	110	18	Good	Animal Alive	3.6	Transplant Site Normal	
280	20	88	18.5	,,	,, ,,	4.2		
244	30	109	28	Fair	,, ,,	5.2		
333	30	30	28	Good	,, ,,			
240	22	118	23.5	,,	,, ,,			
254	34	75	28		Pneumonia	6.2	Patent Pancreatic Duct	Pancreas Normal
292	18	45	17		Perforated Jejunal Ulcer	5.2		
219	20	66	19		Intussusception	5.5		
228	19	38	17		Pneumonia	3.2		
268	17	107	14		,,	4.4		

was performed at periods of 10 to 90 days from the time of transplant and revealed consistently firm union between the pancreas and posterior wall of the stomach. There was no evident atrophy of the remaining pancreas. The gross pancreatic tissue within the lumen of the stomach was digested after 20 to 25 days, but the pancreatic duct remained patent and could be cannulated without difficulty. Serial microscopic sections through the site of the implant demonstrated normal pancreatic gland, unulcerated gastric mucosa and a patent duct orifice. Liver biopsy of 3-10 g taken at the time of exploration was studied chemically and microscopically for lipid deposition. Using the Bloor[7] and modifications of the Kaplan and Chaikoff[8] methods, the fat content of the livers was found to vary from 3.6 to 6.7% in the "transplanted animals" as compared to the normal of 3 to 5%. Microscopic studies of the livers, using special stains, failed to reveal the presence of fatty infiltrations.

In order to study the effect of complete exclusion of the external secretions of the pancreas from the intestinal tract, ligation and division of the pancreatic ducts and partial pancreatectomy was carried out in 5 animals. The results are presented in Chart II.

Although the animals were placed on identical diet as those shown in Chart I they lost weight and exhibited syndrome consisting of anorexia, marked exhaustion, intermittent vomiting and foamy stools. They expired following reëxploration or due to intercurrent infections. The pancreatic lipase was never present in the gastric contents of this series of animals. The blood sugar remained normal. Liver analyses disclosed an increase in the ratio of lipid deposition from 8 to 18.4%. Microscopic sections confirmed the presence of fatty infiltration. They also showed beginning degeneration and atrophy of the cell at the periphery of the lobules. Post mortem examination revealed marked pathological changes in the remaining pancreas. Advanced atrophy with chronic pancreatitis was the outstanding picture; however, in 2 instances acute hemorrhagic pancreatitis and pancreatic abscess resulted from the procedure.

Conclusions. 1. By a modification of the Tripode and Sherwin method, the pancreas can be transplanted into the stomach without danger of immediate acute pancreatitis or peritonitis. 2. The intragastric portion of the transplanted pancreas is eventually digested away. 3. The transplanted pancreas retains its external and internal function and shows no sign of atrophy. 4. Complete ex-

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[7] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

[8] Kaplan, A., and Chaikoff, J. L., *J. Biol. Chem.*, 1935, **108**, 201.

CHART II.

Studies on Dogs with the External Pancreatic Secretion Excluded from the Gastrointestinal Tract by Partial Pancreatectomy and Ligation of the Remaining Pancreatic Duct. (Gastric pancreatic lipase negative, blood sugar normal, sugar in urine negative, liver microscopic fatty infiltration positive.)

Dog No.	Initial wt (lb)	Days after procedure	Wt at death	General health P.O.	Cause of death	Quantitative lipid in liver %	Post-mortem findings	
							Gross	Microscopic
260	33	93	23	Fair	Pneumonia	8.	Pancreatic Abscess Atrophy	Pancreatic Necrosis Chronic Pancreatitis Atrophy
288	28	36	20	Poor	,,	11.2	Atrophy	Chronic Pancreatitis
111	18	33	16	Fair	,,	11.2	,,	Chronic Pancreatitis
295	39	10	36	Poor	Acute Pancreatitis	18.4	Acute Hemorrhagic Pancreatitis	Pancreatic Necrosis
325	32	4	30	,,	,, ,,	10	Acute Pancreatitis	Pancreatic Necrosis

clusion of the external secretion of the pancreas by stated methods produces an abnormal deposition of fat in the liver and concomitant degeneration and atrophy of the liver cells. 5. Lipid deposition and degeneration of the liver does not occur in pancreatic transplants.

10306

Absorption, Acetylation, and Excretion of 2 Sulfanilamido Pyridine (Dagenan, M & B 693).*

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Preliminary reports in English journals[1] on the effectiveness of M & B 693 in pneumonia have been either clinical or bacteriological in nature. Since the administration of this drug will doubtless become widespread in this country, precise knowledge of its absorption and excretion should be made available. Analytical data are herewith presented showing the fate of M & B 693 in both laboratory animals and human patients.

Quantitative estimation of M & B 693 and its conjugated derivative in body fluids is similar to that of sulfanilamide. The original Fuller[2] method was employed using sulfanilamide standards with a factor to account for the difference in molecular weight and color intensity of the pyridine derivative. Determinations of the conjugated form, 2(N-acetylsulfanilamido) pyridine,[3] were similarly made after hydrolysis with p.-toluenesulfonic acid. The percent acetylated values appearing in the tables were calculated from the hydrolyzed values minus the free (unhydrolyzed) times 100.

It was found, as with sulfanilamide, that the rabbit and human detoxify the drug by acetylation; the dog excretes it completely and unchanged in the urine. In all 3 species, the drug is detectable in the blood stream directly after ingestion, and the unchanged, therapeutically active form is completely eliminated from circulation within 24 hours after the last administration, though the drug itself has but limited solubility in water (0.04% at 20°C).

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* Since this was written the name of the drug has been changed officially to sulfapyridine.

[1] *Lancet*, 1938, **1**, 1210, 1230, 1305, 1391, 1402.

[2] *Lancet*, 1937, **232**, 194.

[3] Nomenclature suggested by Crossley, M. L., Northey, E. H., Hultquist, M. E., *J. A. C. S.*, 1938, **60**, 2217.

2(N-acetylsulfanilamido)pyridine has been isolated from both rabbit and human urine. The crystals deposit spontaneously in rabbit and in human urine, occasionally. 1.82 and 2.4 g of the crystalline acetyl derivative were recovered from two 24-hour specimens from a female patient, age 67, given 9.5 g M & B 693 over a period of 3 days Upon one recrystallization from alcohol the M.P. was 226-7°C. Synthetic 2(N-acetylsulfanilamido)pyridine, M.P. 230-231°C. Mixed M.P. 225-227°C.

Analysis: Dumas N found, synthetic, 14.40%, found natural 13.92%, calculated for $C_{13}H_{13}O_3N_3S$ 14.40%.

Dosage: The dosages were varied between the following rough limits: human patients 0.030-0.1 g per kg, per diem; rabbits 0.16-0.64 g per kg; dog 0.20-0.25 g per kg.

Toxic Effects: Laboratory animals show no effects but among patients, nausea is common, often accompanied by mental confusion which passes off upon withdrawal of the drug. Parenteral injection into a rabbit of a solution of the crystalline sodium salt (pH 10.7) was tolerated in a dose 0.4 g per kg but proved lethal at twice that dosage.

Absorption and Elimination of M & B 693. Rabbits: Immediate absorption from the gastrointestinal tract into the blood of rabbits fed varying doses of M & B 693 by stomach tube is shown in the accompanying graphs of 2 typical cases selected from 8. Curves I and II represent the blood levels of unchanged drug at definite time intervals after a single dose of 0.32 g and 0.64 g per kg body weight respectively; curves Ia, IIa, represent the acetylated form. The concentration of M & B 693 reaches a maximum in about 3 hours, begins to fall after 6 or 7, and becomes practically zero after 18-20 hours. Appreciable amounts of the acetylated form still circulate after 26 hours. As far as has been ascertained, however,* this conjugated form is therapeutically inactive, as is N-acetylsulfanilamide. In another experiment on parenteral injection of the sodium salt of M & B 693 of 0.4 g per kg into a rabbit, all of the free drug had disappeared from the blood stream in 11 hours with but 2.5 mg % of the acetylated form remaining.

Dog: 2.0 g M & B 693 was fed to a 12 kg dog in each of 2 separate experiments. Quantitative determinations on the urine showed es-

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* The extreme insolubility of 2-(N-acetylsulfanilamido)-pyridine renders proper testing of the bacteriostatic action against pneumococcus growth difficult. Kindly performed by Miss Hobby on Pn type II.

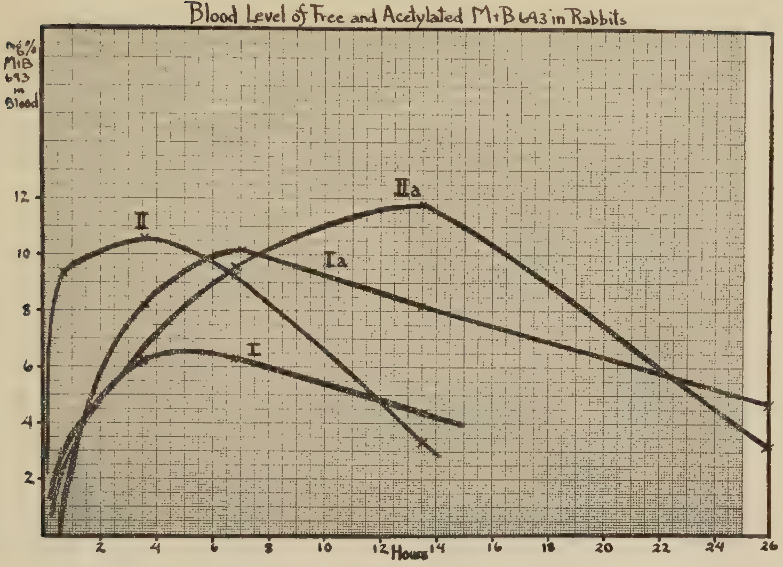

FIG. 1.

Curve I—Blood concentration of free M & B 693 of Rabbit No. 5.49, 3.1 kg, fed 1 g M & B 693.

Curve Ia—Blood concentration of acetylated M & B 693 of Rabbit No. 5.49, 3.1 kg, fed 1 g M & B 693.

Curve II—Blood concentration of free M & B 693 of Rabbit No. 5.48, 3.1 kg, fed 2 g M & B 693.

Curve IIa—Blood concentration of acetylated M & B 693 of Rabbit No. 5.48, 3.1 kg, fed 2 g M & B 693.

sentially complete recovery in 48 hours post administration. None was acetylated. Rates of elimination of M & B 693 were respectively: 13.5 and 21.0% in 4 hours, 71.5 and 73.0% during the next 18 hours, and 6.3 and 8.4% in the next 26 hours, representing a recovery of 98.8 and 94.9%.

Hospitalized Patients: Blood analyses have been made on 75 patients between the ages of 5 and 84 years that had been given a dosage of 2.0-6.0 g M & B 693 per diem over periods varying from 3 to 22 days. In all cases in which no gastrointestinal obstruction was present, rapid absorption into the blood stream was observed and a balance among rates of absorption, acetylation and excretion generally was attained after 24 hours. Accordingly, to insure maximum blood levels, samples for analysis were taken at least 24 hours after the initial dose unless otherwise indicated. Cases 1, 2, 3 (see also footnotes of Table I) show blood concentration of both free and acetylated M & B 693 at the initial phase of drug therapy. Low blood levels and percentage acetylation prevail. Case 4 is characteristic of the final phase. Twenty-four hours after withdrawal of the drug only the acetylated form is present. The remaining cases are typical of the general findings on a group of patients. In case 5 the normal rate of absorption of the drug was modified by a gastrointestinal block which gradually cleared (analyses 2, 3, Case 5). Although this allowed high blood levels, the amount of acetylated product varies but slightly around 50%.

The amount of acetylated (inactivated) drug varies widely in different individuals (30%, cases 1, 6, 7, against 60-80%, case 8), and is somewhat greater than that for sulfanilamide. In general, roughly 50% is acetylated by the human. This is confirmed by urine analysis.

As was to be expected from preliminary rabbit experiments, patients on larger dosages (Cases 1, 7) produced higher blood levels, but the amount and type of circulating drug seems to depend

TABLE I.
Blood Levels of Free and Acetylated M & B 693 in Human Patients.

				M & B 693 Blood Levels		
Case	Patient	Age	Dosage M & B 693 g per diem	Free mg%	Total mg%	Acetylated %
1	Male	27	5.5, 6, 6, 6	5.5	5.5	0*
				10.5	14.2	30
2	Female	58	2, 2.5	2.5†	4.1	39
				1.2	3.3	64
3	”	66	3, 3	2.0‡	2.4	16
				3.7	6.5	43
4	Male	18		0.0	4.0	100§
5	”	74	4, 3.5, 3, 3, 1.5	2.0	3.5	43
				3.5	8.0	56
				5.5	10.5	48
6	Female	16	4, 3.5, 1.5	3.0	4.5	33
				10.5	13.0	19
7	”	40	5, 5, 2	7.5	10.0	25
8	Male	59	3 g for 22 days	1.2	3.0	60
				1.5	8.5	83
				3.0	10.0	70
				2.5	6.2	60
				1.5	6.5	77
				1.0	5.3	81
9	”	71	3 g for 5 days	6.9	14.1	51

*Sample for analysis after 1 g every 4 hr for 20 hr.
†Sample for analysis after 1 g every 4 hr for 10 hr.
‡Sample for analysis after 1 g every 4 hr for 6 hr.
§Sample for analysis 24 hr after last dosage.

on 3 variable factors, (1) rate of absorption, (2) rate of acetylation, (3) rate of elimination of free and acetylated forms. Case 8 is an example in point, and is to be compared with case 9 on the same dosage. In the former, the rapid rate of acetylation may be interpreted as contributing to the low blood levels of the free drug. (Nine urine analyses over a period of 13 days revealed 80-90% of the drug in the acetylated form.)

All of the active form of the drug is removed from general circulation 24 hours after the last dose (Case 4). Urine analyses again bear this out.

TABLE II.
Patient's Excretion of M & B 693 in Urine.

Days	Dosage M & B 693 g per diem	Volume 24 hr specimen cc	Excretion M & B 693			Total daily Excretion M & B 693 g
			Free mg/cc	Total mg/cc	Acetylated %	
1	3					
2	3	800	1.38	2.49	45.0	2.00
3	3	575	3.12	5.90	47.0	3.40
4	3	520	3.50	5.00	30.0	2.60
5	2	245	5.00	7.10	29.6	1.75
6	2	225	6.00	8.50	29.4	1.90
7	0.5	225	2.25	5.35	58.0	1.20
8		360	1.25	1.65	24.0	0.60
9		410	0.17	0.30	43.0	0.12
10		810	0.00	traces		(0.03)
Total	16.5					13.60

Urinary Excretion of Drug: Table II shows the results of daily urine analysis made on a nephrotic youth fed a total of 16.5 g M & B 693 over a period of 7 days. 13.6 g of free and acetylated drug represent a recovery of 82.5%. Both forms had been practically completely excreted 2 days after the last dose. Total drug recovery on 6 other patients varied from 15-85%.

Summary. The concentration of M & B 693 has been determined in the body fluids of the rabbit, dog, and human, following its administration in varying doses. Blood analyses show the appearance of free M & B 693 directly after ingestion; no acetylated form is detectable until later. The percent acetylated is roughly constant for an individual and varies widely around 50% among human subjects. No free drug can be detected in general 24 hours after the last dose and essentially all the acetylated form is eliminated in 48 hours. Urine analyses confirm these findings.

10307

Effect of Testosterone on Pituitary and Mammary Gland.*

R. P. Reece and J. P. Mixner. (Introduced by W. C. Russell.)

From the Department of Dairy Husbandry, New Jersey Agricultural Experiment Station.

The injection of estrogens into ovariectomized[1] or normal female rats[2] increases the lactogen content of their pituitary glands and causes an extension and arborization of the duct system of the mammary gland.[3, 4, 5] The administration of testosterone to spayed rats induces mammary proliferation[5, 6, 7] and marked milk secretion.[6] We were interested, therefore, in determining the influence of testosterone injections on the lactogen content of the pituitary gland.

Thirty-six sexually mature female rats were paired on the basis of body weight. All of the animals were ovariectomized and a mammary gland removed from 4 of the animals. One of each pair was injected subcutaneously daily for 15 days with 200 gamma of testosterone propionate (Oreton†). The other animal of each pair served as a control. Control and experimental animals were sacrificed the day following the last day of injection of the experimental animals. The mammary glands were removed from both groups of animals and studied in whole mounts and in histological sections. The pituitary glands were removed, weighed, and assayed for their lactogen content by injecting the suspended tissue intradermally over the crop glands of common pigeons. Pituitaries from the control and experimental animals were injected into the same group of birds.

Wolfe and Hamilton[8] showed that the injection of 500 gamma of

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* Journal Series Paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

[1] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1937, 266.

[2] Reece, R. P., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 77.

[3] Turner, C. W., *Sex and internal secretions*, 1932, chapter 12 (E. Allen, ed.), Williams and Wilkins Co., Baltimore.

[4] Nelson, W. O., *Physiol. Rev.*, 1936, **16**, 488.

[5] Astwood, E. B., Geschickter, C. F., and Rausch, E. O., *Am. J. Anat.*, 1937, **61**, 373.

[6] Selye, H., McEuen, C. S., and Collip, J. B., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 201.

[7] Nelson, W. O., and Merckel, C. G., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 823.

† The Oreton used in this study was kindly supplied by Dr. E. Schwenk of the Schering Corporation.

[8] Wolfe, J. M., and Hamilton, J. B., *Endocrinology*, 1937, **21**, 603.

testosterone acetate into spayed rats did not alter the weight of the pituitary gland. In our work the dosages employed also failed to alter pituitary weight, the average weight of the pituitary glands from injected animals (10.4 mg) being nearly identical with that of the control animals (10.1 mg). Hamilton and Wolfe[9] also demonstrated that the daily administration of 500 gamma of testosterone propionate reduced the gonadotropic properties of the pituitary gland. In this work the injection of testosterone propionate elicited a 41% increase in the lactogen content of the pituitary glands. Considering the pairs separately, 15 of the pituitaries from the injected rats contained more lactogen than did the glands from the control rats, one was equal to, and 2 contained less lactogen. The fact that the mammary glands of the experimental animals showed evidence of secretory activity suggests that testosterone propionate injections stimulate the pituitary gland to secrete and to discharge into the blood the lactogenic hormone. It would seem, therefore, that the inhibition of lactation in the rat[10] and perhaps in woman[11] by testosterone injections cannot be attributed either to the suppression of lactogen secretion or to the suppression of its discharge into the blood. In contrast to the action of estrogens in bringing about an increase in the lactogen content of the pituitary gland,[1] testosterone propionate augments the lactogen content by increasing the concentration of the hormone in the pituitary tissue and not by inducing an increase in pituitary weight.

The mammary glands of the animals at the time of ovariectomy showed an extensive duct system with numerous end-buds. Signs of involution were present in the mammary glands of the control animals at the time of sacrifice. The mammary glands of the injected animals showed an extensive development of the lobule-alveolar system. This extensive development of the lobule-alveolar system, however, has been brought about not only by hyperplasia but also by hypertrophy inasmuch as secretory activity had been induced in the acini.

Conclusions. The daily injection of 200 gamma of testosterone propionate for 15 days into sexually mature spayed rats augmented the lactogen content of their pituitary glands about 40%, caused no change in pituitary weight, induced an extensive development of the lobule-alveolar system of the mammary glands, and initiated secretory activity. It is suggested that the action of testosterone on the pituitary gland is sufficient to account for the hyperplasia and hypertrophy of the mammary glands of males.

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[9] Hamilton, J. B., and Wolfe, J. M., *Endocrinology*, 1938, **22**, 360.

[10] Folley, S. J., and Kon, S. K., *Proc. Roy. Soc., B*, 1938, **124**, 476.

[11] Kurzrok, R., and O'Connell, C. P., *Endocrinology*, 1938, **23**, 476.

10308

Effect of Insulin on Partially Hypophysectomized Rats.

HERMAN D. JONES. (Introduced by C. S. Robinson.)

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Although much work has been done on the relation of the pituitary gland to the glucose regulatory system, the exact nature of the reaction is not yet clear. Early experiments showed an increased insulin sensitivity in hypophysectomized animals.[1, 9, 5] This has been assumed to indicate the complete ablation of the hypophysis.[7] Posterior lobe extracts have been shown to diminish or abolish this sensitivity and to neutralize the toxic effect of hypoglycemia.[1, 8] Some workers have shown that the nature of the antagonism of posterior lobe extract to insulin-glucose mechanism is related to the peripheral utilization of blood glucose,[2] while others have sought to explain this antagonism, existing in over-activity of the pituitary, by changes in general carbohydrate metabolism within the animal organism.[6]

Sex hormone extract from the anterior lobe of the hypophysis has produced an increased blood glucose, while the growth hormone has been shown to produce little effect.[3] Other investigators could demonstrate little or no effect of anterior lobe products on this insulin-glucose mechanism.[8]

Hypophysectomy has been believed to disturb the regulatory mechanism which tends to preserve glycogen during fasting.[4] An accessory pancreatic factor has been postulated, since the complete ablation of the hypophysis in depancreatized dogs enables them to utilize carbohydrate.[8] The magnitude of fall in blood glucose values after insulin has been shown to be increased from 14% to

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[2] Cohen, H., and Libman, B., *Quart. J. Med.*, 1937, **6**, 157.

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[5] Geiling, E. M. K., Campbell, D., and Ishikawa, Y., *J. Pharm. and Exp. Therap.*, 1927, **31**, 247.

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[7] Hartman, C., Firor, W. M., and Geiling, E. M. K., *Am. J. Physiol.*, 1930, **95**, 662.

[8] Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511.

[9] Houssay, B. A., and Magenta, M. A., *Compt. Rend. Soc. Biol.*, 1925, **92**, 822.

35% by complete hypophysectomy in monkeys, while incomplete hypophysectomy produced an almost insignificant change from normal.[12]

Experimental investigation in glandular physiology has been done largely on completely hypophysectomized animals. Therefore, it seemed desirable in this investigation to study the nature of insulin sensitivity of albino rats partially hypophysectomized.

Hypophysectomies were done according to a modification of Smith's[11] technic and blood sugars were determined by Miller-Van Slyke's[10] microtitration procedure. The 47 strong, active rats used were allowed at least 2 weeks to recover from the hypophysectomy. Obviously, a standard dose of insulin was required to produce an appreciable hypoglycemia but not shock or convulsions. This, determined experimentally, was found to be ¼ clinical unit per 125 g body weight for normals, and 1/16 clinical unit per 125 g body weight for hypophysectomized rats. A 0.2 cc blood sample was taken immediately before insulin injection and every 30 minutes afterwards for 2 hours, then at hourly intervals for the third and fourth hours, when the animals were sacrificed and autopsied immediately to determine the degree of completeness of the hypophysectomy. In doubtful cases the sella was sectioned serially and studied for pituitary fragments. Twenty-two animals showed complete hypophysectomy, and hypoglycemia with a general trend of the value downward. Twenty-five showed incomplete hypophysectomy, with a hyperglycemia in each reaching its height at 1½ to 2 hours. No blood sugar fell as low in this group as in the former one.

It was observed that 5 of the 22 animals of the completely hypophysectomized group died before the termination of the experiment. These animals went into typical insulin shock with convulsions and died. No attempt was made to relieve the hypoglycemic condition. The blood glucose values in Table I are typical of both completely and partially hypophysectomized rats and give the results obtained on animals 27, 52, and 61, respectively. In 10 of the 25 animals which were incompletely hypophysectomized there was an initial fall in blood glucose 30 minutes after insulin injection. Fifteen showed an increase or remained at about the initial level. After reaching the peak at ½ to 2 hours the value began to fall and either returned to about the initial level or fell slightly below. Histological or gross findings proved all these animals to have some part of the gland present, the amount varying from fragments up to as much as 3 mg. Further investigations are now in progress in an effort to determine what portion of the gland produced this phenomenon.

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[10] Miller, Benjamin F., and Van Slyke, Donald D., *J. Biol. Chem.*, 1936, **114**, 583.

[11] Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

[12] Smith, P. E., Tyndall, H. H., Dotti, L., and Engle, E. T., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 247.

TABLE I.

Blood Glucose in Completely and Partially Hypophysectomized Rats After Insulin Injection in mg per 100 cc of Blood.

	Animal No.	Normal Glucose	Minutes after injection of insulin					
			30	60	90	120	180	240
Completely hypophysectomized	27	125	100	73	71	74	56	65
Incompletely hypophysectomized	52	112	98	106	118	120	108	86
	61	112	128	134	140	164	136	118

The evidence here presented affords ample proof of a difference in insulin response of partially and completely hypophysectomized rats. Results confirm the recorded statements that removal of the entire hypophysis renders an animal hypersensitive to insulin.[9, 5] They show that hyperglycemia is produced after insulin in partially hypophysectomized rats, and that the maximum fall in blood glucose, after the peak is reached, is never so great as in those completely hypophysectomized.

Smith, *et al.*,[12] demonstrated an initial fall in blood glucose in 3 partially hypophysectomized monkeys after a 30-minute interval following insulin. My results indicate that after 30 minutes 10 animals showed an initial fall, in agreement with their findings, while 15 showed an initial rise. It would be of interest to know what would have been the trend of blood glucose curves in their animals if the experiment had been continued over a longer period.

The results suggest that the pituitary fragments are stimulated in some manner so that they secrete a substance not only antagonistic to insulin but producing a mobilization of glucose in the blood stream following insulin injections. The exact biological nature of this mechanism cannot yet be elucidated. These results suggest a method which might prove beneficial clinically in determining pituitary deficiency and the degree of hypophysectomy in experimental animals.

The author wishes to acknowledge the courtesy of Dr. John C. Burch, who placed at his disposal all the hypophysectomized rats needed for this investigation.

10309 P

Complement-Fixation in Infections with the Virus of Lymphocytic Choriomeningitis.

J. E. Smadel, R. D. Baird and M. J. Wall. (Introduced by T. M. Rivers.)

From the Hospital of The Rockefeller Institute of Medical Research, New York City.

The demonstration by Howitt[1] of complement-fixation in the presence of mixtures of suspensions of guinea-pig brain infected with the virus of lymphocytic choriomeningitis and hyperimmune sera from guinea pigs suggested a simple serological method of diagnosing the malady in man and lower animals. We conduct the test as follows: 0.2 cc each of antigen, inactivated immune serum, and diluted guinea-pig serum (2 units of complement), are mixed and incubated overnight at 3°C. Two units of hemolysin (0.2 cc) and 0.5 cc of a 5% suspension of washed sheep-erythrocytes are added. Tests are read after ½ hour at 37°C.

Complement-fixing antigen occurs in spleen, consolidated lung, liver and brain of acutely ill guinea pigs infected with the virus, but not in tissues from normal animals. The antigen is most readily obtainable from spleen and least from brain; splenic suspensions containing 1 part (dry weight) in 6000 give complete fixation. Ten percent suspensions of fresh organs are run at 30,000 rpm for 20 minutes in the concentration-centrifuge[2] and the supernatant fluid is filtered through a Seitz pad. Such preparations of spleen are not anticomplementary after storage; the contained antigen is little affected by storage at 3°C for 6 months, by heating at 56°C for ½ hour, by variations in pH range from 4.5 to 9, or by dialysis. Antigen has been obtained from guinea pigs infected with 4 strains of virus maintained in this laboratory and from mice inoculated with the strain most commonly used.

Centrifugation at 30,000 rpm for 20 minutes sediments the virus but leaves the complement-fixing antigen in the supernatant fluid. The virus can be repeatedly washed without appreciable loss of infectivity, provided the suspending medium contains 2% normal serum. Ultra-sediment, washed 2-4 times, diluted to the initial volume, and centrifuged at 10,000 rpm for 10 minutes without appreciably sedimenting the virus, gives a supernatant fluid that is not anticomplementary, and, although infective in dilutions of 10^{-6}, rarely fixes more than partially in dilutions of 1:2 and 1:4. On the other hand, Seitz filtrates of the supernatant fluid from the original ultracentrifugation, possessing infective titers of 10^{-1} or less, contain as much complement-fixing antigen as do filtrates of the original splenic suspension and give complete fixation when diluted 1:8 to 1:32. This indicates that the complement-fixing antigen in infected tissue can be separated from the virus. Thus, a soluble substance appears to be associated with this virus infection as well as with bacteriophagy, influenza, psittacosis, vaccinia, yellow fever, and myxomatosis.[3]

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[1] Howitt, B. F., *J. Immunol.*, 1937, **33**, 235.

[2] Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1936, **64**, 503.

Howitt[1] concluded that the reaction is specific for lymphocytic choriomeningitis, since she found no fixation in cross-tests with materials from animals infected with the viruses of St. Louis encephalitis, and several types of equine encephalomyelitis. We have also found no evidence of fixation in cross-tests with materials from vaccinia, myxomatosis, and influenza.* We have found that guinea pigs develop complement-fixing antibodies before their sera neutralize the virus; the former are usually demonstrable 2 weeks after infection, while the neutralizing antibodies appear in 4-5 weeks and are present in appreciable amounts by the 6th week. Furthermore, the former reach a peak (titer 1:64-1:512) at about 5-6 weeks, and then begin to decline.

We have tested a number of sera from patients with lymphocytic choriomeningitis.[4, 5] Many of the original specimens were found to be anticomplementary after storage; nevertheless, sera from 6 patients taken 6-8 weeks after onset of the disease were suitable and gave positive fixation in the presence of lymphocytic-choriomeningitis antigen. Moreover, the sample taken at the onset of meningitis from one of these individuals failed to fix complement. Blood drawn 1½ to 3 years after the disease still neutralized the virus in all but one instance, but none of the samples definitely fixed complement. Twelve patients who failed to develop neutralizing antibodies in their sera following benign lymphocytic meningitis also failed to develop complement-fixing antibodies. In addition, human sera known to give positive Wassermann reactions or fixation with gonococcal antigen failed to react with lymphocytic-choriomeningitis antigen. Lepine and Sautter[6] have also demonstrated complement-fixing antibodies in the serum of a laboratory worker recovered from infection with the virus of lymphocytic choriomeningitis.

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[3] Rivers, T. M., Ward, S. M., and Smadel, J. E., *J. Exp. Med.*, 1939, **69**, 31.

* Influenzal antiserum and antigen were provided by Dr. Frank L. Horsfall.

[4] Scott, T. F. McN., and Rivers, T. M., *J. Exp. Med.*, 1936, **63**, 397.

[5] Baird, R. D., and Rivers, T. M., *Am. J. Pub. Health*, 1938, **28**, 47.

[6] Lepine, P., and Sautter, V., *Ann. Inst. Pasteur*, 1938, **61**, 519.

Summary. A soluble specific antigen, which occurs in tissues of animals infected with the virus of lymphocytic choriomeningitis, fixes complement in the presence of immune serum; the virus freed from the soluble antigen fixes complement poorly or not at all. Patients recovered from lymphocytic choriomeningitis develop in their serum antibodies that react with the soluble antigen to fix complement.

10310

Sulfanilamidopyridine (2-Para-aminobenzenesulfonamidopyridine) in Experimental Infections with Type XXII Pneumococcus.*†

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Our recent observations[1] indicated that sulfanilamide had definite therapeutic properties in experimental pneumococcus infections in mice, and that the effectiveness of the drug varied with the type of pneumococcus used as the infecting agent. A marked curative action was obtained in infections with 19 of the 30 types of pneumococci (I, V, VII, IX, X, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XXI, XXIII, XXV, XXVIII, XXXI, and XXXII); in infections with the remaining 11 types (II, III, IV, VI A and B, VIII, XI, XX, XXII, XXIV, XXVII, and XXIX) the drug prolonged life but did not lead to recovery.

Recently, Whitby[2] reported that sulfanilamidopyridine (2-para-aminobenzenesulfonamidopyridine) had curative properties when administered to mice infected with Types I, VII and VIII pneumococci and prolonged life in infections with types II, III and V. In experiments on Type I, he found that sulfanilamidopyridine was a more effective therapeutic agent than sulfanilamide. This observa-

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* Supported in part by a grant from the Union Central Life Insurance Company, Cincinnati, Ohio.

† Sulfanilamidopyridine was generously supplied by Dr. David A. Bryce, The Calco Chemical Company, Bound Brook, New Jersey.

[1] Schmidt, L. H., and Hilles, C., in press.

[2] Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

tion suggested that sulfanilamidopyridine might have a favorable therapeutic action in infections due to those types of pneumococci which were refractory to sulfanilamide therapy. This has been investigated; the results in Type XXII infections are reported here.

Therapeutic Properties of Sulfanilamidopyridine. In the initial experiment 2 groups of mice were infected intraperitoneally with 10^{-6} cc of a 12-hour blood broth culture of a Type XXII pneumococcus; this quantity of culture contained approximately 200 pneumococci and was equivalent to 100 minimum lethal doses.‡ The first group (10 mice) served as untreated controls. The second group (20 mice) was treated with sulfanilamidopyridine in a manner similar to that described by Whitby; 20 mg of the drug were administered, orally, 2 hours after infection and 6, 12 and 18 hours thereafter, then at 24-hour intervals for 5 successive days. Cultures of tail blood were made at the conclusion of treatment; the mice were observed closely for 24 additional days; survivors were sacrificed then, and cultures of heart blood were made. The results are summarized in Fig. 1 (Groups 1 and 2).

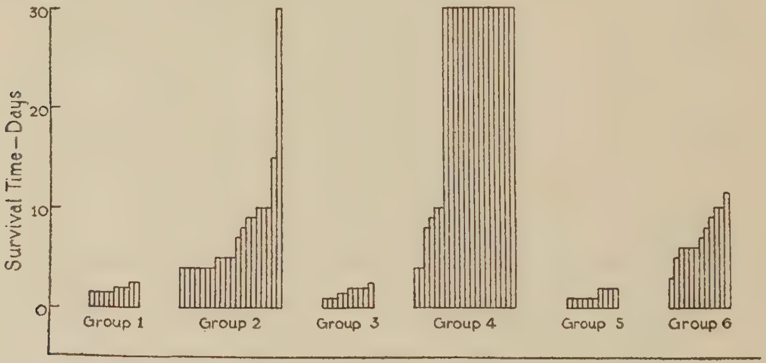

FIG. 1.

Therapeutic Properties of Sulfanilamidopyridine and Sulfanilamide in Experimental Infections with Type XXII Pneumococcus.

Each column represents one mouse.

Groups 1, 3 and 5: Untreated controls.

Group 2: Sulfanilamidopyridine administered orally; 20 mg 2 hours after infection and 6, 12 and 18 hours thereafter, then at 24-hour intervals for 5 succeeding days.

Group 4: Sulfanilamidopyridine administered orally; 20 mg 2 hours after infection and every 6 hours thereafter for 4 days, then at 12-hour intervals for 2 days.

Group 6: Sulfanilamide injected subcutaneously; 10 mg 2 hours after infection and every 6 hours thereafter for 8 days.

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‡ The stock culture of this pneumococcus was obtained from Miss Annabel Walter, Bureau of Laboratories, Department of Health, New York City. The procedure for increasing the virulence of organisms and determining the minimum lethal dose has been described elsewhere.[1]

Treatment with sulfanilamidopyridine, in the above manner, prolonged life but led to recovery in only one of the 20 mice. All of the treated animals survived 4 days, and 9 lived 6 days or longer, whereas all the untreated controls died within 60 hours after infection. Seven of the 9 mice that survived 6 days or more had positive blood cultures at the time therapy was discontinued, and yet 6 of these 7 mice lived an additional 3 to 9 days. These results were not significantly better than the results obtained with sulfanilamide (Fig. 1, Groups 5 and 6).

In a subsequent experiment, sulfanilamidopyridine was administered at more frequent intervals. Mice were infected with 100 lethal doses of Type XXII pneumococcus as in the preceding experiment; 20 mg of sulfanilamidopyridine were administered 2 hours after infection and every 6 hours thereafter for 4 days, then at 12-hour intervals for 2 additional days. Fourteen of the 20 mice so treated recovered (Fig. 1, Groups 3 and 4). Of the 6 mice that did not recover, 2 lived 4 days; of the remaining 4, one died on the 8th, one on the 9th and 2 on the 10th day after infection—all died after therapy had been discontinued.

This experiment shows that sulfanilamidopyridine has definite curative properties in infections with type XXII pneumococcus. The fact that more intensive therapy was required in Type XXII infections than in Types I and VII is of interest, for in our previous experiments[1] it was noted that the dosage of sulfanilamide required for effective treatment varied in infections with different types of pneumococci.

Mode of Action of Sulfanilamidopyridine. Four mice in the last experiment described—those that died after therapy had been discontinued—had positive blood cultures on the 6th day after infection. Cultures from 2 of these mice contained typical Type XXII pneumococci. The culture from the third animal contained Gram positive diplococci which were bile soluble and gave a negative Neufeld reaction when mixed with antipneumococcus sera Types I to XXXII. The culture from the 4th mouse contained both the typical Type XXII pneumococci and the diplococci giving a negative Neufeld reaction. This last culture has been studied in some detail.

Pure cultures of the "Neufeld positive" and "Neufeld negative" organisms were obtained—both fermented inulin and were bile soluble. There were marked differences in the growth of these organisms on blood agar. The colonies of the "Neufeld positive" organisms were smooth—as were those of the stock culture; the colonies of the "Neufeld negative" organisms were rough.

The "Neufeld negative" organisms were passed through mice; after 2 successive passages they gave a positive Neufeld reaction. The "Quellung" at this stage was not as marked as that of the stock Type XXII pneumococcus; after 5 successive passages, the "Quellung" reaction of the originally "Neufeld negative" organisms was identical with that of the stock XXII.

Observations by Whitby,[2] and Telling and Oliver[3] have suggested that sulfanilamidopyridine exerts a definite action on the capsules of Types I and III pneumococci. Our observations on Type XXII pneumococcus offer further evidence that sulfanilamidopyridine therapy leads to production of decapsulated pneumococci. Neither our own observations nor those of Whitby and Telling indicate whether this is the result of capsular degeneration or inhibition of capsule formation.

The effect of sulfanilamidopyridine on the pneumococcus capsule may partially explain the therapeutic properties of the drug. It is generally agreed that decapsulated pneumococci are readily attacked by the phagocytes of the host and for that reason are less virulent than capsulated forms. That the "Neufeld negative" organisms were relatively avirulent is indicated by the following experiment.

A group of 30 mice was infected intraperitoneally with 100 M.L.D. of the stock Type XXII pneumococcus (160 organisms by plate count); a second group was infected with 150 "Neufeld positive" organisms isolated from the 4th mouse mentioned above; the third group received 150 "Neufeld negative" organisms isolated from the same mouse. Mice from each of these groups were sacrificed at 6, 12 and 24 hours after infection; in addition, mice from the third group were killed at 48, 96 and 144 hours. Cultures and smears of peritoneal washings were made; heart blood samples were taken and the number of organisms therein determined by means of pour plates.

TABLE I.

Comparison of *In Vivo* Growth of Stock Type XXII Pneumococcus with That of "Neufeld Positive" and "Neufeld Negative" Type XXII Pneumococci Isolated from a Mouse Treated with Sulfanilamidopyridine.

	No. of pneumococci per cc of blood Hr after infection			Cultures from peritoneal washings Hr after infection		
Infecting Organisms	6	12	24	6	12	24
"Stock"	23×10^3	13×10^4	32×10^6	+	+	+
"Neufeld Positive"	28×10^3	31×10^4	140×10^6	+	+	+
"Neufeld Negative"	0	0	0	—	—	—

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[3] Telling, M., and Oliver, W. A., *Lancet*, 1938, **1**, 1391.

The data in Table I show that the stock XXII pneumococci and the "Neufeld positive" organisms (those obtained from the sulfanilamidopyridine treated mouse) invaded the blood stream quickly and multiplied equally well. "Neufeld negative" organisms could not be cultured from the blood or peritoneal washings at any time.

Summary. Experimental infections with Type XXII pneumococcus, which are refractory to sulfanilamide therapy, can be treated satisfactorily with sulfanilamidopyridine. The amount of drug required for effective treatment is larger than that necessary in Types I, VII, and VIII infections (Whitby). Avirulent decapsulated Type XXII pneumococci have been isolated from the blood of mice treated with sulfanilamidopyridine. This supports the suggestion of Whitby, and Telling and Oliver that the drug exerts a definite action on the pneumococcus capsule.

10311

Sulfanilamide in the Treatment of Experimental Trypanosomiasis of Rats.

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The reputed effectiveness of sulfanilamide in the treatment of malaria induced us to determine whether or not the compound was therapeutically effective in the treatment of trypanosomiasis of rats. Buttle, Gray and Stephenson[1] were unsuccessful with sulfanilamide therapy against malaria in canaries and *Trypanosoma equiperdum* infections of mice.

Sixteen white rats, weighing from 150 to 170 g, were inoculated intraperitoneally with approximately 500,000 *Trypanosoma equiperdum;* 24 hours later some showed a few trypanosomes in the tail blood. Treatment was started at this time, 4 receiving 0.08 g per kilo by intravenous injection 24, 30, 48, 54, 72, and 78 hours after inoculation; 4 were given 0.160 g and 4 given 0.200 g per kilo at the same intervals. Four were kept as untreated controls and all died of trypanosomiasis 4 to 5 days after inoculation; 3 additional animals were kept as drug controls and survived 8 days when the experiment was terminated.

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[1] Buttle, G. A. H., Gray, W. H., and Stephenson, D., *Lancet,* 1936, **1**, 1286.

The results summarized in Table I indicate that sulfanilamide was completely ineffective as all treated animals succumbed to trypanosomiasis in 4 to 7 days in spite of the very large doses given, corresponding to as much as 4.8 to 12.0 g per 60 kilos for 6 doses in succession at intervals of 6 to 18 hours. The low toxicity of sul-

TABLE I.
Sulfanilamide by Intramuscular Injection in the Treatment of Rats Inoculated with *T. equiperdum.*

Rat No.	Dose, per kilo, g*	Exam. tail blood for trypanosomes; days after inoc.							
		1	2	3	4	5	6	7	8
1	.08	+§	++	++++	++++	D			
2	”	—	+	+++	++++	++++	++++	D	
3	”	+	++	++++	++++	D			
4	”	—	++	++	++++	++++	++++	D	
5	.160	+	++	++++	D				
6	”	+	++	++++	D				
7	”	—	++	++++	++++	D			
8	”	—	++	+++	++++	++++	D		
9	.200	+	++	++++	D				
10	”	+	++	++++	D				
11	”	—	++	++++	++++	D			
12	”	—	++	++++	++++	++++	D		
13†	0	+	++	++++	D				
14	0	—	++	++++	++++	D			
15	0	+	++	++++	D				
16	0	—	++	++++	++++	++++	D		
17‡	.08	—	—	—	—	—	—	—	—
18	.160	—	—	—	—	—	—	—	—
19	.200	—	—	—	—	—	—	—	—

*24, 30, 48, 54, 72 and 78 hours after intraperitoneal inoculation with 500,000 *Trypanosoma equiperdum.*

†Rats 13, 14, 15 and 16 infection controls; no treatment.

‡Rats 17, 18 and 19 drug controls.

§+ = about 5 to 10 per field; ++ = 10 to 20 per field; ++++ = very large numbers; — = survived; D = died.

TABLE II.
Sulfanilamide by Oral Administration in the Treatment of Rats Inoculated with *T. equiperdum.*

Rat No.	Dose per kilo, g*	Tryp. tail blood; days after inoculation					
		1	2	3	4	5	6
1	.45	+†	+	++	++++	D	
2	.47	—	+	+	++	++++	D
3	.49	+	+	++	++++	D	
4	.5	+	—	++	++++	D	
5	.35	—	+	++	++++	++++	D
6	.2	—	+	++	++++	D	
7	Control	+	+	++	++++	D	
8	”	—	+	++	++++	D	

*24, 30, 48, 72 and 78 hours after intraperitoneal inoculation with 500,000 *Trypanosoma equiperdum.*

†+ = about 5 to 10 per field; ++ = 10 to 20 per field; ++++ = very large numbers; D = died.

fanilamide for rats by intravenous injection is indicated by the survival of the drug controls.

Eight additional animals were inoculated intraperitoneally with approximately 500,000 *Trypanosoma equiperdum;* 2 untreated controls died on the fifth day after inoculation. The remaining 6 animals were given capsules of sulfanilamide powder in dose of 0.2 to 0.5 g per kilo 24, 30, 48, 72, and 78 hours after inoculation (Table II). All succumbed to trypanosomiasis in 5 to 6 days after inoculation, the doses corresponding to as much as 12.0 to 30.0 g per kilo of weight.

Summary. Sulfanilamide by intravenous and oral administration was ineffective in the treatment of *Trypanosoma equiperdum* infections of rats.

10312

Estrus-Inhibiting Effects of Inanition

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The effects of complete and partial starvation upon the estrous cycle of adult female rats were studied. Smears from the vaginal mucosa were taken for at least 2 weeks before any experimental procedures were instituted. Seven groups of 5 rats each were used. One group was kept as control; 2 groups received no food or water, and another only water at the onset of starvation; the fifth group was starved intermittently so that the loss of weight was more gradual. The remaining 2 groups were starved completely without water until they died. Each animal was weighed daily. The water and food consumption of the groups fed was recorded daily. It was found that the animals in all the starved groups eventually showed complete inhibition of the estrous cycle, and that the inhibition was roughly related to the loss in body weight, as shown in the table. When the inhibition of the estrous cycle was complete, all but 2 groups were fed again, with resulting return to the normal cyclical changes in the vaginal mucosa.

Experiments to Determine the Factors Involved. In analyzing the estrus-inhibiting effects of starvation, one must distinguish between

TABLE I.
Estrus-Inhibiting Properties of Starvation Inanition.

Rat Group	Procedure instituted	Resulting anestrus in—days	Loss in Weight		Subsequent treatment	Effect on vaginal mucosa	Weight loss at end of experiment	
			%	Days			%	Days
St-25	Complete starvation	1-4	30	6	Continued starvation plus estrogen	Estrus in 3 days	40	11
St-26-7	Complete starvation	1-3	30	6	Continued starvation	Continued anestrus	42	11
St-19	Complete starvation	1-5	33	7	Refed	Estrus in 7 to 13 days	None	21
St-28	Complete starvation	2-4	25	5	Continued starvation plus Follutein	Estrus in 3 days	33	8
St-21	Complete starvation plus water	1-5	30	7	Refed	Estrus in 4 to 9 days	None	16
St-22	Chronic inanition	3-6	15	7	Refed	Estrus in 3 to 6 days	None	
St-20	Control	None	—	—	—	—	Gained 13	21

an inability of the vaginal mucosa to respond to estrogenic hormone; and the possibility that the ovaries have ceased to produce the hormone during the period of inanition. If the latter be true, one must further distinguish between a primary failure of the ovary, and a failure of the pituitary gland to produce the gonadotropic hormone. It is conceivable that any or all of these factors may be involved.

1. *The effect of estradiol monobenzoate upon the inanition anestrus.* Two groups of 10 rats were starved completely for 6 days. On the day starvation was begun 4 of the 10 animals were in estrus; 3 were just entering the diestrous phase; 2 were in diestrus; and 1 was in proestrus. The 4 in estrus completed their cycles and then entered permanent diestrus or anestrus. The 3 which were entering diestrus remained in anestrus throughout. Of the 2 rats in diestrus, 1 remained so, the other went as far as the nucleated epithelial stage on the second and third day of starvation and then developed anestrus. The animals in proestrus showed a normal cycle which ended in anestrus. These 10 rats were then divided into 2 groups on the sixth day of starvation. At this time, most of them had been in the anestrus for 4 days. One group was kept as an untreated control, while the rats of the other group were injected daily with 3 R.U. of estradiol monobenzoate (Progynon-B)* subcutaneously. The 5 untreated control rats continued in complete anestrus to the 11th day of starvation, when they were autopsied. The gonads were weighed and fixed for sectioning, as were the other glands of internal secretion. The 5 rats which received estrogen remained in anestrus for 24 hours, but on the 2nd day showed nucleated epithelial cells. On the 3rd day of injection and on the 9th day of starvation, all were in typical estrus and continued so until autopsy on the 11th day.

Two groups of 5 rats each were starved acutely except that one received drinking water. Their estrous behavior resembled that of the rats analyzed above. They were refed on the 7th day of starvation, when the percentage of body weight lost averaged 33, and all the rats had been in anestrus for 1 to 5 days. Five to 8 days following refeeding, when the animals had regained the loss in weight, a return to normal estrous cycles was evidenced. Two weeks after refeeding was begun, and all the rats were showing normal cycles, daily injections of 2 R.U. of estrogen were begun. Eleven days later the rats were starved as before, but due to the daily injections of estrogen they continued to have cycles as though no starvation had been instituted. The control group of rats showed normal estrous cycles during these experiments.

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* We are indebted to Drs. Gregory Stragnell and Erwin Schwenk of the Schering Corporation for their supply of Progynon-B.

Another group was underfed by allowing food freely for 24 hours after 48 hours of fasting. On the 8th day, when the rats had lost an average of 15% of their body weight, they were in anestrus for from 3 to 6 days. Upon being refed all rats continued anestrous from 3 to 6 days, when they resumed their normal cycles. This experiment was repeated 2 weeks later. This time the rats received daily injections of 2 R.U. of estrogen. The results confirm the impression gained from the above experiments. Animals that are acutely starved or chronically underfed will cease to have estrus when the body weight has dropped but some 15%. These rats may be made to have estrus again by the subcutaneous injection of estrogen even when 40% of the body weight has been lost. The presence of estrogen in the body prevents the appearance of this "inanition anestrus."

2. *The effect of gonadotropic hormone upon inanition anestrus.* A group of 5 rats was examined and then starved acutely. After 5 days of starvation with ensuing anestrus, the rats were injected with 3 to 4 R.U. of gonadotropic hormone (Follutein)† daily for 3 days. Forty-eight hours after the first injection the vaginal smear of 1 of the rats showed nucleated epithelial cells. In 72 hours (8th day of starvation) all 5 animals were in full estrus.

The lack of estrogen in the animal in sufficient quantities to activate the vaginal mucosa during the course of inanition is evidently due to diminished production of gonadotropic hormone, rather than to any inability of the ovary to respond to the hormone. No quantitative data are as yet available to warrant a statement as to any altered sensitivity of the ovary.

Discussion. It has been found that such diverse factors as double adrenalectomy,[1-5] dietary deficiencies,[6, 7] especially B_1-avitaminosis,[8] poisoning with dinitrophenol, thyroid extract[9-13] result in the loss of estrous cycles in rats. Analysis of these data indicated an underlying common factor, namely, the loss in weight in all of these experimental animals. Corroborative evidence against the assumption of some more specific cause of the anestrus was furnished by the fact that estrous activity was resumed when the weight lost had been regained. On the other hand, insulin injections,[14] manganese-free diets,[15] morphine,[16] and nicotine[17] poisonings in rats, procedures which resulted in no disturbance of nutrition in no way affected the estrous cycle. Our own experiments support the conclusion that in the investigations alluded to above, the loss of estrus was either wholly or at least in part due to the concomitant inanition. The inanition appears to produce anestrus through changes induced in the hypophysis.

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† We are indebted to Drs. G. A. Harrop and John Anderson of E. R. Squibb & Sons for their supply of pituitary-like hormone.

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10313

Progesterone Will Maintain Adrenalectomized Rats.*

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Certain observations suggest the possibility that progesterone is capable of maintaining the adrenalectomized animal. (a) There is a chemical similarity in the structure of progesterone and certain of the hormones found in the adrenal cortex.[1, 2] (b) 21-hydroxyprogesterone (desoxycorticosterone) will maintain the adrenalecto-

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* Supported in part by a grant from the Josiah Macy, Jr., Foundation.

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mized animal.[3] (c) Life is maintained in adrenalectomized animals with functional corpora lutea, as in pregnancy or pseudopregnancy.[4, 5, 6] (d) Crystalline progesterone is shown to have a sodium retaining action in the adrenalectomized dog which is similar to that exhibited by the cortical hormones.[7, 8]

On the basis of these findings, it would seem that progesterone should maintain life in adrenalectomized animals. Various observers, however, report evidence to suggest that such is not the case.[4, 9, 10] It is possible, nevertheless, that inability to record such a phenomenon is due to insufficient dosage, and the following study was undertaken to test this hypothesis.

While this paper was in the process of being written, Gaunt, *et al.*,[11] have presented data showing that progesterone maintained life in 9 out of 19 adrenalectomized animals in dosages of 1-2 mg per day.

Procedure. Litter mate, male animals of our stock colony of albino rats were bilaterally adrenalectomized by the lumbar route. The average weight at operation was 41.4 g, the extremes being 36 and 49 g. Each litter was divided into treated and control groups which were kept under identical conditions. The diet consisted of Purina Fox Chow and tap water, supplemented on the first 3 days with powdered milk.

All animals were weighed once daily until death. The treated animals were given 4.0 mg of crystalline progesterone† in oil at the time of weighing, for the first 15 days, starting the day of the operation, some of the control animals being injected with plain oil. The criteria of complete adrenalectomy or absence of accessory adrenal tissue were lack of weight gain and subsequent death. However, the few animals which died within 48 hours of the operation have not been included in this series, as we feel that death in these instances can not be attributed to adrenal insufficiency.

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† We wish to thank Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products for the generous supply of progesterone.

Results. The 11 control animals lived an average of 5.1 days (minimum 3 days, maximum 7 days). The average daily weight change for this group is shown in the graph (Fig. 1).

The 10 animals, treated for 15 days with progesterone, lived an average of 18.0 days (minimum 11 days, maximum 23 days). One of the treated animals, showing a weight gain, died on the eleventh day of unknown cause. The other treated animals all showed a gain in weight. Following the cessation of treatment, these animals lived on an average of 4.76 days (extreme 2 to 9 days). The average daily weight change is shown in the graph (Fig. 1).

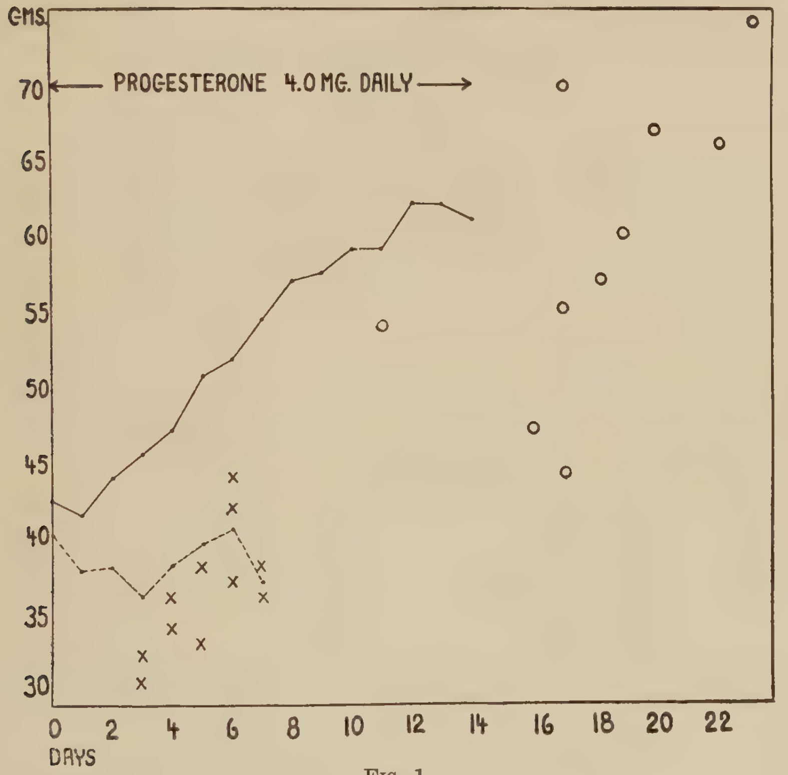

FIG. 1.

Average weights of treated (———) and control animals (............). "X" shows weight on last day of life of control animal. "O" shows weight on last day of life of treated animal. Note that the smaller animals died sooner after cessation of treatment.

From the graph, it can readily be seen that 4.0 mg of crystalline progesterone per day will maintain life and a gain in weight of immature adrenalectomized male rats. When treatment was discontinued, weight gain ceased and the animals died of adrenal insufficiency. On the basis of these findings it may be concluded that the negative results reported in the past were due to insufficient dosage.

Conclusions. Progesterone is "cortin-like" in that it will maintain life and weight gain in immature adrenalectomized male rats.

10314 P

Selective Bacteriostatic Action of Sodium Lauryl Sulfate and of "Dreft."

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The bacteriostatic action of various soaps has undergone a considerable amount of study by many investigators. Recently, a somewhat related class of substances, the sodium alkyl sulfates, has been synthesized. These substances have also been made use of as detergents in such preparations as "Dreft," "Drene" and other commercial products.

The alkyl sulfates are half-esters of sulfuric acid. In this respect they differ from soaps which are combinations of fatty acids and alkalis. In both cases, however, the alkyl chain probably determines its bactericidal effectiveness. Sodium lauryl sulfate is a sodium alkyl sulfate containing 12 carbon atoms.

The similarity of these substances to soaps suggested the possibility that similar bacteriostatic action might be exhibited by them. Accordingly, sodium lauryl sulfate as such, and "Dreft" which contains a large amount of sodium lauryl sulfate were tested for their bacteriostatic action. Twenty-one strains of Gram positive bacteria, 20 strains of Gram negative bacteria and 6 strains of common molds were used in these studies. In general, all tests were made on Difco standard nutrient agar to which the test substances had been added in varying amounts so as to make concentrations of 1.0% sodium lauryl sulfate and of 1.0, 0.5, 0.25, and 0.1% "Dreft". The plates were streaked with heavy suspensions of the organisms and then incubated at appropriate temperatures for 48 hours.

The effects of sodium lauryl sulfate and of "Dreft" on the growth of the 41 strains of bacteria and 6 strains of molds studied in this investigation are shown in Table I. It is evident that the Gram positive organisms were prevented from growing, whereas the

TABLE I.
Bacteriostatic Action of Sodium Lauryl Sulfate and of "Dreft."*

	Sodium lauryl sulfate	"Dreft"			
	Concentration in nutrient agar				
	1%	1%	.5%	.25%	.1%
Gram positive					
Sarcina lutea	—	—	—	—	—
Gaffkya tetragena	—	—	—	—	—
Staphylococcus aureus (12 different strains)	—	—	—	—	—
" *albus*	—	—	—	—	—
" *citreus*	—	—	—	—	—
Bacillus subtilis	—	—	—	—	—
" *anthracis*	—	—	—	—	—
" *mesentericus*	—	—	—	—	—
" *mycoides*	—	—	—	—	—
" *vulgatus*	—	—	—	—	—
Gram negative					
Neisseria catarrhalis	—	—	—	—	—
Klebsiella pneumoniæ	+	+	+	+	+
Serratia marcescens	+	+	+	+	+
Achromobacter liquefaciens	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+
" *fluorescens*	+	+	+	+	+
Aerobacter aerogenes	+	+	+	+	+
Proteus vulgaris	+	+	+	+	+
Alcaligenes fecalis	—	—	—	—	+
Escherichia coli	+	+	+	+	+
" *communior*	+	+	+	+	+
Salmonella paratyphi	+	+	+	+	+
" *schottmülleri*	+	+	+	+	+
" *aertrycke*	+	+	+	+	+
" *enteritidis*	+	+	+	+	+
" *gallinarum*	+	+	+	+	+
" *pullorum*	+	+	+	+	+
Eberthella typhosa	+	+	+	+	+
Shigella dysenteriæ	+	+	+	+	+
Vibrio comma	—	—	—	—	—
Molds					
Aspergillus sp.	—	—	—	—	—
" *niger*	—	—	—	—	—
Mucor sp.	—	—	—	—	—
Penicillum sp.	—	—	—	—	—
Rhizopus sp. (—)	—	—	—	—	—
" " (+)	—	—	—	—	—

+ = growth; — = no growth.
*All control inoculations on nutrient agar alone resulted in luxuriant growth.

Gram negative bacteria grew as freely as on the nutrient agar controls. There were 3 exceptions to the latter statement: *Vibrio comma, Alcaligenes fecalis,* and *Neisseria catarrhalis* were definitely inhibited in their growth. It was found, however, that *A. fecalis* was able to grow in the concentration of 0.1% "Dreft".

Difficulty was encountered when an attempt was made to determine if there existed a concentration of "Dreft" greater than 1%

capable of inhibiting the growth of the Gram negative bacteria. In concentrations of 10% and above, a reaction between "Dreft" and the Difco nutrient agar often occurred which resulted in a heavy white precipitate, and such agar did not solidify properly. Determinations made by the cup-plate method showed that *Eberthella typhosa* especially, and very probably other Gram negative bacteria, were not inhibited by even 10% and 15% concentrations of "Dreft".

The selective bacteriostatic properties of sodium lauryl sulfate and "Dreft" suggest their use in selective media. This has already been proposed by Cowles[1] with respect to the presumptive test in sanitary water analysis. Perhaps even greater use of such a selective medium may be made in the study of intestinal disturbances where the presence of Gram positive organisms in the stools is frequently confusing. The fact that *A. fecalis* is inhibited almost as much as Gram positive organisms may simplify such examinations where the isolation of this bacterium is of no importance.

While our work was in progress, a paper by Cowles[2] appeared, in which are reported results essentially the same as ours. Cowles' work differed from ours, however, in several respects. For the test medium this author used nutrient broth, whereas nutrient agar was used in our work. In place of "Dreft", as used in our studies, Cowles used a liquid form of this product which is known commercially as "Drene". Work with 27 bacterial species was reported in Cowles' paper, and our work has so far dealt with 47 strains of 35 species of bacteria and molds. Cowles' additional important contribution was to point out the greater bacteriostatic action against *Staphylococcus aureus* by sodium alkyl sulfates as the number of carbon atoms in the alkyl chain is increased.

From the experiments here recounted, it is apparent that sodium alkyl sulfate and the commercial preparation "Dreft" possess a high degree of bacteriostatic selectivity. The growth of Gram positive bacteria and molds was definitely inhibited while most Gram negative bacteria grew freely in the presence of these substances in nutrient agar.

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[2] Cowles, P. B., *Yale J. Biol. and Med.*, 1938, **11**, 33.

10315 P

Lack of Antianemic Principle in Human Liver from Case of Carcinoma of Stomach.

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(Introduced by Kenneth L. Burdon.)

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The relationship between gastric secretion and hematopoiesis is now generally accepted.[1, 2, 3] There is also general agreement that the hematopoietic principle is formed by the stomach and stored in the liver.[4, 5] After total gastrectomy the antianemic principle of hog's liver is depleted.[6, 7] The most active antianemic principle is obtained from the mucosa of the pyloric antrum.[8, 9]

Recently there has been demonstrated the absence of the "intrinsic factor" in the gastric juice of a patient with macrocytic anemia, who was shown by roentgenologic examination to have a scirrhous carcinoma of the stomach.[10] Ventriculin was used as a control therapy. We have tested the antianemic potency of an extract prepared from the liver of a patient whose death was due to scirrhous carcinoma of the pyloric and prepyloric regions of the stomach. As control therapy we used an extract prepared from the normal liver of another patient whose death was caused by a cerebral hemorrhage.

In a patient with carcinoma of the stomach the necropsy was performed one hour after death. A scirrhous carcinoma was found involving the entire pyloric and prepyloric region. Histologic study showed that the entire mucosa of this portion of the stomach had been replaced by neoplastic growth. There was no evidence of visceral metastasis.

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[1] Castle, *Am. J. Med. Sci.*, 1929, **178**, 748.

[2] Sturgis and Isaacs, *J. A. M. A.*, 1929, **93**, 747.

[3] Castle and Ham, *J. A. M. A.*, 1936, **107**, 1456.

[4] Morris, Schiff, Burger and Sherman, *J. A. M. A.*, 1932, **98**, 1080.

[5] Greenspon, *J. A. M. A.*, 1936, **106**, 266.

[6] Bence, *Z. f. klin. Med.*, 1933, **126**, 127.

[7] Goodman, Geiger and Claiborn, Proc. Soc. Exp. Biol. and Med., 1935, **32**, 810.

[8] Henning and Brugsch, *Deutsche Med. Wochenschr.*, 1931, **57**, 757.

[9] Meulengracht, *Z. f. klin. Med.*, 1936, **130**, 468.

[10] Goldhamer, *Am. J. Med. Sci.*, 1938, **195**, 17.

At necropsy the liver and gastrointestinal tract of the patient who died of cerebral hemorrhage revealed no abnormalities.

The extracts from both livers were made according to the Lilly method (Solution Liver Extract—Lilly, N.N.R., 1937, p. 320), under exactly the same circumstances. The extracts, after having been passed through a Jenkins filter, were cultured and found to be sterile. When they were used experimentally on anesthetized dogs no significant quantities of either pressor or depressor substances were found.

The patient used for the determination of the presence of the antianemic principle had a typical Addisonian pernicious anemia in relapse, with nervous symptoms. Hematologic examination revealed 1,740,000 red blood cells and 4,800 white blood cells per cu mm, and 7.8 g or 56% hemoglobin (Hellige). The mean corpuscular volume was 132 cubic microns and the mean corpuscular hemoglobin was 45 micromicrograms. The percentage of reticulocytes was 1.1. Achlorhydria was persistent following the administration of histamine.

The intramuscular injection of 9 cc of liver extract, representing 126 g of liver from the patient with carcinoma of the stomach, failed to produce an increase in the reticulocyte count over a period of 9 days. On the 10th day after the first injection, the intramuscular injection of 9 cc of the control extract, representing 133 g of liver from the patient with cerebral hemorrhage, caused a marked elevation of the number of reticulocytes, which reached a peak of 22.0% on the 6th day after the second injection. By the 11th day the number of red blood cells had increased to 2,650,000 and the hemoglobin value had increased to 78%. The total period of observation was 20 days.

Summary. An extract was prepared from a human liver obtained from a patient whose death was due to carcinoma of the pyloric and prepyloric regions of the stomach. Its administration to a patient with Addisonian pernicious anemia in relapse failed to produce a reticulocytic response. A control extract was prepared from the liver of a patient whose death was due to cerebral hemorrhage. Its administration to the same patient caused a marked stimulation of hematopoiesis. It is of interest that the portions of gastric mucosa which were replaced by neoplastic tissue in the patient with carcinoma of the stomach were those which, according to experimental observations, are the most active in the production of the "intrinsic factor."

10316

Relation of Hypophysis to Spleen. III. Hypophysectomy and Resistance to *Trypanosoma lewisi.*

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Hypophysectomized animals are less resistant to the effects of anesthetics, drugs, toxins and spontaneous infections (Perla[1]). The decrease in general resistance is associated with involutional changes in the suprarenal glands. The alteration in natural resistance to poisons such as histamine[2] is attributed in part to the withdrawal of the adrenotropic hormone of the hypophysis, since it may be restored by the administration of suprarenal cortical hormone (Perla[3]), emulsions or extracts of dried anterior hypophysis rich in adrenotropic factor[4] and heated acid extracts containing only active adrenotropic hormone.[5]

The removal of the hypophysis is associated with a drop in natural resistance to both spontaneous bacterial and to induced protozoan infection *(Trypanosoma lewisi)* (Perla,[1] Culbertson and Molomut[6]). A drop in resistance to infection of this type is in part due to the effect of hypophysectomy on the spleen and lymphoid tissues (Perla[5]). Perla and Marmorston and Taliaferro, Cannon and Goodloe[7] observed that removal of the spleen in *Bartonella*-carrier or *Bartonella*-free rats disturbs the production of trypanolysins and reproduction-inhibitory antibodies and a more severe and more prolonged trypanosome infection occurs. Suprarenalectomy, on the other hand, lowers the resistance but does not alter the course of the infection in those surviving the toxemia (Perla and Marmorston[9]). Since removal of the hypophysis results in atrophy of splenic and lymphoid tissue it seemed reasonable to anticipate an alteration in response to infection with *Trypanosoma lewisi.** It was suggested (Perla[8]) from experimental evidence that a spleen-stimulating factor exists in the anterior hypophysis. In the present communication further data on the effect of hypophysectomy in rats on infection with *T. lewisi* is presented. The effects of extracts of the pituitary was determined both on hypophysectomized infected and unoperated infected animals.

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[1] Perla, David, *Proc. Assn. for Research in Nervous and Mental Diseases,* 1936a, **17**, 471.

[2] Perla, D., and Rosen, S. H., *Arch. Path.*, 1935, **20**, 222.

[3] Perla, David, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 797.

[4] Perla, David, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 121.

[5] Perla, David, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 751.

[6] Culbertson, J. T., and Molomut, N., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 28.

[7] Taliaferro, W. H., Cannon, P. R., and Goodloe, S., *Am. J. Hyg.*, 1931, **14**, 1.

[9] Perla, David, and Marmorston, J., *The Spleen and Resistance,* Williams and Wilkins, 1935, Baltimore.

In the experiments of Culbertson and Molomut[6] all the hypophysectomized rats succumbed in an average period of 5.4 days after injection of the trypanosomes.† The intensity of infection, however, did not exceed that of the controls as determined by the number of trypanosomes at the peak of infection. Some of the operated controls also died of the infection and only a few were protected by anterior pituitary extract (Squibb, in amount of 10 rat units daily‡).

Methods: All the rats were of Bartonella-free stock, 6 to 8 weeks of age. They were injected 2 to 3 weeks after hypophysectomy with 50,000 trypanosomes. Some were treated with daily injections of acid or alkaline extracts of dried anterior pituitary substance from the day of operation.

TABLE I.

Effect of Complete and Partial Hypophysectomy in the Rat on Course of Infection with *Trypanosoma lewisi* (All infected 2 to 3 weeks after hypophysectomy).

Operation	No. rats	Treatment	Avg No. tryp. at peak	No. days from injec. of tryp. to peak	Duration of infection in days
Complete hypophysectomy	10	None	741,000	7.3	27.4
Partial hypophysectomy	11	"	526,000	7.5	18.7
Unoperated controls	10	"	524,200	6.8	19.2
Complete hypophysectomy	10	Alkaline extr. of ant. lobe*	573,500	6.2	18.8
Complete hypophysectomy	7	Acid extr. of ant. lobe†	1,126,000	7.8	26.8

*The alkaline extracts were prepared as follows: 10 gm of dried powder was extracted with 100 cc of 0.5 N sodium hydroxide over night. The fluid was brought up to pH 7.8, centrifuged and filtered through a Zeiss filter. 1 cc of this extract was injected daily intraperitoneally.

†The acid extract of dried anterior hypophysis was prepared by the method of Loeb.[5] 10 g of dried powder was extracted with 100 cc of ½% acetic acid over night. The fluid was brought to pH 6.8, centrifuged and then brought to 7.8 and filtered through a Zeiss filter.

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* The evidence of the relation of the spleen to resistance is reviewed in a monograph by Perla and Marmorston.[9]

[8] Perla, David, *J. Exp. Med.*, 1936, **63**, 599.

† All received 1 million trypanosomes intraperitoneally.

‡ The nature of the rat unitage is not mentioned.

Results: From Table I, it is seen that the infection in the hypophysectomized animals extended for a period 50% longer in duration than that in either the unoperated rats or the partially hypophysectomized animals. Partial hypophysectomy had no effect on the course of the infection. The intensity of the infection as determined by the number of trypanosomes at the peak of the disease was somewhat greater in the hypophysectomized rats than in those of the control groups with intact hypophysis. None of the animals died.

The duration of infection in the hypophysectomized rats treated with alkaline extracts was approximately the same as in the control groups. The duration of the infection in the hypophysectomized rats treated with acid extracts was as long as that of the untreated hypophysectomized rats. The intensity of the infection as estimated by the number of trypanosomes at the peak was more severe in the rats treated with acid extract than in those of all the other groups.

The number of days from the day of injection to the height of infection was approximately the same in all groups. Reproduction forms were not present in the blood after the first 8 to 10 days in any group. From these observations it is probable that the reproduction-inhibiting factor, ablastin, was normally produced in the hypophysectomized animals. The prolongation of the infection following hypophysectomy suggests some impairment in the production or activity of trypanolysins. When a rat does not form ablastin in infections with *T. lewisi,* the parasite produces a lethal infection.§ Splenectomy in animals does not abolish the formation of ablastin but in some instances only it may inhibit its production. It does, however, prolong the duration of the disease.[9] Since hypophysectomy decreases splenic activity and growth but does not completely abolish its function, the effect of hypophysectomy need not be as severe as splenectomy on the course of infection with *T. lewisi.*

The results reported by Culbertson and Molomut[6] may be attributed to the fact that a much greater infecting dose of trypanosomes was employed in their experiments and the disease, therefore, may have proved fatal. The animals, in their experiments, were infected within one week after operation at a time when the resistance of such animals is known to be lowest (Perla and Rosen[2]). In the present work the animals were not infected until 2 to 3 weeks after operation at a time when the general resistance is not as severely affected but the spleen and lymphoid tissue have undergone pronounced atrophy (Perla[8]). The results of these experiments is further proof of the effect of hypophysectomy on the functional response of splenoid tissue.

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§ In Culbertson's experiments, though death occurred, it cannot be concluded that ablastin was not produced, since the rats died prior to the time when the peak of infection occurs in control rats. Further, the number of trypanosomes at the time of death did not exceed that of the controls. In other words, death was not necessarily an expression of impairment of immune processes but of enhanced susceptibility to the toxic effects of infection.

Summary and Conclusions. Hypophysectomy in rats was followed by a decrease in resistance to a protozoan infection such as *Trypanosoma lewisi.* The infection was prolonged and the intensity of the infection exceeded that of the controls. The administration of alkaline extracts of anterior lobe, rich in spleen-stimulating factor restored the resistance to the normal level.

It is likely that the altered resistance to protozoan infection in hypophysectomized rats is in part attributed to impairment of the function of the splenoid tissue.

10317 P

Comparative Experimental Studies on Symptomatology and Anatomical Changes Produced by Anoxic and Insulin Shock.

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Thirty-eight rabbits were exposed to anoxia for short periods during which the oxygen percentage in the mixture with nitrogen was so slowly decreased as to avoid excitement, though in some instances the anoxia was prolonged until respiration ceased. This slow deprivation of oxygen is termed anoxic shock. The rabbits were exposed to from 1 to 51 anoxic shocks during a period of from 1 to 60 days. The duration of a single shock varied from 15 to 60 minutes. Twenty-nine other rabbits were injected with insulin sufficient to produce coma and convulsions.

The symptoms of the insulin and anoxic shock were similar except that those due to anoxia occurred on a more rapid time scale. Anoxia was characterized by increase of the respiratory rate, dilatation of the ear arteries, muscular weakness, somnolence and finally decrease of the respiratory rate and complete cessation of respiration, though the heart continued beating. In insulin shock muscular weakness and somnolence also occurred before convulsions and coma. Here, too, respiratory arrest was observed. With anoxia 2 types of convulsions might be distinguished. A too rapid diminution of the oxygen percentage precipitated convulsions. A more interesting type of convulsion was observed following the resaturation of the blood with oxygen. Characteristically these convulsions could be suppressed by lowering the oxygen percentage again.

The anatomical changes produced by both types of shock, the anoxic and insulin shock, were also much the same in the central nervous system as well as in the heart, skeletal musculature and liver. In the central nervous system they consisted of from slight to severe degenerations of ganglion cells and reactive glial proliferation. The frequency of shocks, and in the insulin rabbits the individual susceptibility and the severity of the clinical response to the administered insulin were the decisive factors on which depended the severity of the anatomical changes. These changes were especially impressive wherever large pyramidal cells were present, but were by no means confined to them. The cerebral cortex, the Purkinje cells in the cerebellum, the cornu Ammonis, the medulla and the spinal cord were usually definitely involved. No particular nuclei were specifically affected, but more or less numerous ganglion cells in various areas. In a few instances that had shown exceptionally severe clinical responses almost all ganglion cells of circumscribed areas of the cortex or the cornu Ammonis had perished, as described in experiments of Stief and Tokay.[1] An adverse vascular reaction during seizures might be answerable for these localized damages. The more evenly distributed changes were similar to those recently reported by Weil, *et al.*[2] In insulin shock they are best explained by the deprivation of the ganglion cells of carbohydrate which is, according to Himwich, *et al.*,[3] their foremost nutritional material; in anoxic shock by the lack of oxygen[4] which is equally indispensable for the maintenance of their metabolism. Though the same ganglion cells were adversely affected either by insulin or anoxic shock, there was, nevertheless, a difference in the morphological appearance of those cells. The ganglion cells severely affected by insulin shock faded away leaving a "bleached" area in stained preparations;

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[1] Stief, A., and Tokay, L., *Z. f. d. ges. Neurol. u. Psych.*, 1932, **139**, 434.

[2] Weil, A. E., Liebert, E., and Heilbrunn, G., *Arch. Neurol. and Psychiat.*, 1938, **39**, 467.

[3] Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., *Science*, 1937, **86**, 271.

[4] Himwich, H. E., Bowman, K. M., Fazekas, J. F., and Orenstein, L. L., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 359.

in anoxic shock they were shrunken and darkly stained, as if they were calcified.

Both types of shock produced in the cardiac musculature smaller or larger areas composed of pale staining swollen muscle cells. Only in exceptional cases were a few muscle cells definitely destroyed as was also indicated by the formation of reactive cellular proliferation. Its absence in most of the animals even if they had been frequently treated evidenced the reversibility of the changes described above. The skeletal musculature showed the same changes, but to a more marked degree, rupture of muscle fibres being visible in the iliopsoas muscle and in the musculature of the thigh. Here a definite reactive cellular proliferation was also noted. In the liver hydropic changes of hepatic cells were observed after exposure to insulin or anoxic shocks. Definite focal necroses of the liver were found only in those rabbits which had shown severe responses to insulin or anoxic shock. Fatty infiltration in the parenchymatous organs was not noted following anoxic shock, obviously because of the relatively short duration of a single shock period.

The hydropic changes in the heart, skeletal musculature and liver are usually reversible. In the brain, however, single ganglion cells of various areas are destroyed, and this perhaps is a necessary step in the effective therapy of schizophrenia. The great similarity of the symptomatology and anatomical changes produced either by insulin or anoxic shock might warrant a test as to the curative results obtainable by anoxic shock in schizophrenia.

10318

The Antigonadotropic Factor. The Quantitative Aspects of the Prolan-Antiprolan Reaction.

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From the Laboratory of the Obstetrical-Gynecological Department of the Rothschild-Hadassah Hospital, Jerusalem.

The question as to whether or not the neutralization of prolan by antiprolan is subject to quantitative laws is of interest since it permits of certain conclusions in regard to the mechanism of this reaction. We know that fermentive processes are not subject to quantitative laws. On the other hand, adsorptive processes (*e. g.*, the toxin-antitoxin reaction) are so, subject to the deviation known as Danysz's phenomenon. This phenomenon means: if a certain quantity of toxin is added to a certain quantity of antitoxin the mixture is more toxic if the toxin is added by a fractional method than if it were added at one dose. If, however, the reverse process is followed and the antitoxin is added to the toxin by this method the toxicity of the mixture is not greater than if the antitoxin were added all at once. Bordet described the same phenomenon in connection with hemolysins and Craw with agglutinins.

Following the technic indicated by Zondek and Sulman[1] we investigated whether Danysz's effect is also apparent in connection with the prolan-antiprolan reaction. In the first series of experiments we added prolan to antiprolan by the fractional method, in the second we followed the reverse method, adding antiprolan to prolan. The experimental order was chosen so that each time 10 RU of prolan or 10 PAU of antiprolan* were dissolved in 0.5 cc of water. The prolan powder had the titer of 1 mg = 100 RU, the antiprolan powder that of 1 mg = 1 PAU. We added the substance at intervals of 2 hours. During the whole experimental period, lasting 8 hours, the substances were kept in the incubator.

The experimental order is shown in Table I.

TABLE I.

1st series: Prolan is added to antiprolan.

Inf. rats R	Antiprolan PAU	Prolan RU	Method of addition	Gonadotropic reaction
1	30	30	1 dose	—
2	30	40	1 ”	+
3	30	30	3 ”	—
4	30	40	4 ”	+

2nd series: Antiprolan is added to prolan.

Inf. rats R	Prolan RU	Antiprolan PAU	Method of addition	Gonadotropic reaction
5	40	30	1 dose	+
6	40	40	1 ”	—
7	40	30	3 ”	+
8	40	40	4 ”	—

Table I shows that the neutralizing effect of prolan and antiprolan is subject to strictly quantitative laws. Even if we use 4 portions of 10 units each in every instance 40 units are neutralized, independent of whether prolan is added to antiprolan or the reverse.

The fact that the quantitative neutralization of prolan and antiprolan follows strict laws differentiates this reaction from fermentive reactions and from most of the immune reactions. Even if we consider antiprolan formation *in vivo* as a process of immunization, yet antiprolan is distinguished from the other groups of immune bodies, which have hitherto been known, by the quantitative behavior of its *in vitro* reaction. The absence of the usual serologic reactions of prolan and antiprolan *in vitro* also demonstrates that we are considering a group of immune bodies of an exceptional type.

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[1] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

* 1 PAU = 1 prolan anti-unit is the smallest amount of antiprolan able to annihilate the gonadotropic effect of 1 RU of prolan.

10319

Progonadotropic Augmentary Immune Sera after Protracted Injection of Hypophyseal Gonadotropic Hormone in Rabbits.

FELIX SULMAN AND ABRAHAM HOCHMAN. (Introduced by Bernhard Zondek.)

From the Laboratory of the Obstetrical-Gynecological Department of the Rothschild-Hadassah Hospital, Jerusalem.

Collip[1] as well as Thompson[2] showed that the injection of gonadotropic extracts of the pituitary gland occasionally led to the production of immune sera which augmented the gonadotropic effect of the antigen. Rowlands[3] was able to confirm these results. These synergistic sera exert their progonadotropic action exclusively on the gonadotropic extracts of hypophyseal origin (prosylan), not however on hormones derived from pregnancy blood or pregnancy urine (prolan). Thompson, therefore, explains the effect of the progonadotropic sera as acting through the formation of an augmentary factor, which is formed as an antihormone against Evans' antagonist[4] of the pituitary. The progonadotropic effect is invariably replaced by the anti-gonadotropic effect during the course of the immunization process. It is by no means the rule that the augmentary factor is produced following injection of gonadotropic extracts of hypophyseal origin. Its formation is, as a matter of fact, subject to laws which have hitherto been unknown.

In 14 cases in which we used protracted immunization with gonadotropic extracts from pregnancy blood, pregnant mare's blood, and pregnancy urine we have so far not detected the presence of a progonadotropic serum either in rabbits or in male goats. When, however, we injected 2 rabbits several times with hypophyseal extracts from rats, we found, in one case, a serum which showed a progonadotropic instead of an antigonadotropic effect.

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[1] Collip, J. B., *Canad. Med. Assn. J.*, 1937, **36**, 199.

[2] Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 640.

[3] Rowlands, I. W., *Proc. Roy. Soc. London*, 1938, Ser. B, No. 837, **124**, 492.

[4] Evans, H. M., Pencharz, R. I., and Simpson, M. E., *Univ. Calif. Publ. Anat.*, 1936, **1**, 237.

The 2 rabbits had daily been injected subcutaneously with the pituitary glands of 2 rats extracted with 2 cc of distilled water. After 5 months' immunization one of the rabbits showed the progonadotropic effect. The technic of the titration was that indicated by Zondek and Sulman[5] in prolan A units after 2 hours' incubation of antiserum and hypophyseal extract.

Infantile female rats 3-4 weeks old, weighing 25 to 30 g were given in 3 series of experiments: (1) Aqueous extracts of hypophyseal glands of rats, 4 cc = 1 RU. (2) Increasing quantities of progonadotropic serum. (3) A mixture of extracts of the hypophyseal glands of rats and progonadotropic serum after 2 hours' incubation.

TABLE I.

	Inf. rats	Hypophyseal extr.	Progon. serum	Gonadotropic reaction
		cc RU	cc	
1st series	R 1	0.5 = 0.125	—	—
	R 2	1.0 = 0.25	—	—
	R 3	2.0 = 0.5	—	—
	R 4	3.0 = 0.75	—	—
	R 5	4.0 = 1.0	—	+
2nd series	R 6	—	0.025	—
	R 7	—	0.05	—
	R 8	—	0.1	—
	R 9	—	0.5	—
	R10	—	1.0	—
3rd series	R11	0.5 = 0.125	0.025	+
	R12	0.5 = 0.125	0.05	+
	R13	0.5 = 0.125	0.1	+
	R14	0.5 = 0.125	0.5	+
	R15	0.5 = 0.125	1.0	+

Table I shows the progonadotropic effect. It shows that as small a quantity as 0.025 cc of progonadotropic serum is able to increase the gonadotropic effect of 0.5 cc = 0.125 RU of hypophyseal extract, which amount is below the threshold, so that it equals the effect of 4 cc = 1 RU. Thus the gonadotropic effect of the hypophyseal extract of rats can be multiplied by 8. Control experiments proved that the addition of normal rabbit serum was unable to increase the gonadotropic titer of hypophyseal extracts.

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[5] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 708.

Moreover, the augmentary effect could be obtained exclusively in connection with extracts derived from the pituitaries of rats, not with those of men.

Summary. Following protracted injection in 16 rabbits of gonadotropic hormone derived from different sources we succeeded in one case in producing a progonadotropic serum, after treatment consisting of daily injections for a period of 5 months. 0.025 cc of this serum was able to multiply by 8 the gonadotropic effect of an aqueous extract of rat pituitaries. Control experiments showed that this augmentary effect was specific: it was neither obtained with normal serum acting on extracts of the rat pituitary, nor with progonadotropic serum acting on gonadotropic hormones of a different origin, *viz.*, of human pituitaries.

10320 P

Allergy in *Brucella* Infections.

PABLO MORALES-OTERO AND LUIS M. GONZÁLEZ. (Introduced by D. H. Cook.)

From the School of Tropical Medicine, University of Puerto Rico, under the auspices of Columbia University.

Cutaneous hypersensitiveness to specific antigen as a test for *Brucella* infection has been used by several authors.[1-11] Unfortunately, the diversity of preparations used as antigen makes correlation of results difficult. As a rule, a positive reaction has been considered as denoting past or present infection.

Recently, the authors[12] published a method for the preparation of

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[1] Fleischner, E. C., and Meyer, K. F., *Am. J. Dis. Children*, 1918, **16**, 268.

[2] Burnet, E. T., *Arch. Inst. Pasteur del Afrique du Nord*, 1922, **2**, 187.

[3] Taylor, R. M., Lisbonne, M., and Vidal, L. F., *Mouvement Sanitaire*, 1935, **12**, 51.

[4] Dubois, Ch., and Sollier, N., *Annals Inst. Pasteur*, 1931, **47**, 311.

[5] Bastai, P., and Rotta, C., *Policlinico*, 1928, **35**, 393.

[6] Levin, W., *J. Lab. and Clin. Med.*, 1930, **16**, 275.

[7] Trenti, E., *Policlinico*, 1925, **32**, 867.

[8] Mitra, M., *Pediatria*, 1924, **32**, 721.

[9] Bua, F., *Minerva Medica*, 1926, **6**, 394.

[10] Giordiano, A. S., *J. Am. Med. Assn.*, 1929, **93**, 1957.

[11] Huddleson, I. F., Johnson, H. W., and Hamann, E. E., *Am. J. Public Health*, 1933, **23**, 917.

[12] Morales Otero, P., and Gonzalez, L. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 703.

a purified protein-antigen from *Brucella* and decided to use the preparation to investigate cutaneous hypersensitiveness to *Brucella* among milkers and cattle handlers in farms near the city of San Juan, P. R., where endemic abortion in cattle is prevalent. The method used was as follows: The desired amount of the purified protein produced from the *Brucella* organism is accurately weighed and suspended in a few cc of saline; then enough drops of N/10 hydroxide solution are added until a clear solution is obtained, and this is brought back to neutrality with N/10 HCl. The resulting solution is diluted with saline so that it may contain 0.1 mg per cc. By means of a sterile, tuberculin-type syringe and a 26-gauge needle, 0.1 cc of the diluted protein-solution is injected into the skin over the flexor surface of the forearm. The site of the injection is examined 48 hours later. In subjects that react positively, there is marked erythema and edematous induration. As a rule, the area of edema is much larger than the erythematous area. The size of the reaction varies with its intensity and in some cases there is a constitutional reaction shown by general malaise and slight fever, with pain or itching in the injected arm.

Two hundred and twelve adults occupied as milkers or cattle-handlers were tested. At the same time, 15 cc of blood were drawn and agglutination, complement-fixation, and opsono-cytophagic tests for *Brucella* were made. Of the 212 cases tested, 7 or 3.3% showed a positive complement-fixation, 5 or 2.3% gave a positive agglutinative reaction, 46 or 21.7% gave a positive opsono-cytophagic reaction, and 63 or 29.6% showed hypersensitiveness. Of these, 17 or 8% had constitutional reaction as shown by pain in the arm, general malaise, and slight fever, usually lasting 24 hours.

Of all the persons tested, 7 or 3.3% had a history of undulant fever. The reactions in this group are shown in Table I.

Of 7 persons working in our department, 4 were negative to all tests and 3 reacted as follows: One of us (P.M.O.), who has been

TABLE I.

Name	Skin-reaction*	Agglutination	Complement fixation*	Opsono-cytophagic index
F.G.	+++	Negative	++++	Moderate
J.R.	+	1-450	Negative	Slight
J.A.	Negative	1-450	++++	Negative
M.A.M.	+++	Negative	++++	"
F.S.	Negative	1-1600	+++	Marked
J.M.	"	1-1600	++++	Negative
C.R.	+	1-3200	+	"

*The purified *Brucella* protein was used as antigen. In grading the skin reaction the method recommended by Aronson[13] was followed.

[13] Aronson, J. D., *Am. Rev. Tuberc.*, 1934, **30**, 727.

handling *Brucella* cultures for the last 10 years, gave a positive (3+) cutaneous reaction and a weak positive (2+) complement-fixation, while the reactions in the opsono-cytophagic and agglutinative tests were negative. The other (L.M.G.), who has been working with us for 5 years, gave a positive (3+) cutaneous reaction, a positive (3+) complement-fixation, a positive opsono-cytophagic reaction and a negative agglutination. In the third (R.C.), who has been in contact with cultures for about 2 years, all 4 reactions were positive. Those 3 of us giving positive reactions are the only ones handling *Brucella* cultures, and we have never shown signs or symptoms of the disease.

The results show that there is no correlation between cutaneous allergy to *Brucella* and other immunological reactions, such as agglutination, complement-fixation and opsono-cytophagic index. As in Evans' report,[14] in our series 15 cases that were positive to the opsono-cytophagic index, gave a negative cutaneous reaction, while 18 showing a positive cutaneous reaction were absolutely negative to the opsono-cytophagic test.

Contact with infected material causes the development of a state of hypersensitiveness as shown by injection of the purified *Brucella* protein, although signs or symptoms of the disease may not have been evidenced. This has been explained by the fact that hypersensitiveness usually appears later than agglutination and complement-fixation and that it lasts longer. However, in the light of recent experiments by Sabin,[15] the possibility of sensitization without infection by coming in contact with infected material through abraded skin is worthy of careful consideration. The cutaneous test should be of value in epidemiological investigations, but until further work is done, the cutaneous reaction should not be considered as proof of present or past infection, since it only shows the development of a state of hypersensitiveness in persons that have been in close contact with infected material.

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[14] Evans, A. C., Robinson, F. H., and Baumgartner, L., *U. S. Public Health Rep.*, 1938, **53**, 1507.

[15] Sabin, F. R., and Joyner, A. L., *J. Exp. Med.*, 1938, **68**, 659.

10321

A Note on the Differences Between Histamine and Leukotaxine.

VALY MENKIN.

*From the Department of Pathology, Harvard University Medical School, Boston.**

Previous studies have demonstrated the presence in inflammatory exudates of a factor capable of increasing capillary permeability.[1] Purification of the exudate material has yielded a crystalline nitrogenous substance which is extremely active in inducing prompt increased capillary permeability as shown by the pronounced seepage of an intravenously injected vital stain into treated cutaneous tissue.[2] Furthermore, this material manifests a chemotactic effect. Within 15 to 30 minutes following its intracutaneous inoculation, polymorphonuclear leukocytes accumulate in abundance in the lumen of small vessels. These cells at first adhere closely to the endothelial wall from which they soon migrate actively into the extra-capillary spaces.[3] The chemotactic property of this active substance may be demonstrated *in vitro*. Polymorphonuclear leukocytes in a sample of exudate readily aggregate around particles of the material deposited on a slide.[3] For the sake of convenience this substance has been tentatively named *leukotaxine*.[4] The untreated inflammatory exudate contains both a permeability and a chemotactic factor. Prolonged chemical fractionation yields a relatively homogeneous crystalline end-product which still manifests both of the properties possessed by the original exudate. The analytical procedure has thus failed to dissociate the two factors. The available data warrant, for the sake of convenience, a name for this active substance which *per se* appears to behave as a definite biological unit. Leukotaxine is thus capable of rapidly reproducing the basic sequences of the inflammatory reaction, namely an initial increased capillary permeability followed by polymorphonuclear leukocytic infiltration.

In the foregoing studies, no concrete evidence has been obtained to support Lewis' view that a histamine-like substance in exudates or in their partially purified active fractions, can adequately account for the primary mechanism of increased capillary permeability in

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* Aided by grants from the Milton Fund of Harvard University, from the International Cancer Foundation, and from the Academy of Arts and Sciences.

[1] Menkin, V., *J. Exp. Med.*, 1936, **64**, 485.

[2] Menkin, V., *J. Exp. Med.*, 1938, **67**, 129.

[3] Menkin, V., *J. Exp. Med.*, 1938, **67**, 145.

[4] Menkin, V., *Arch. Path.*, 1937, **24**, 65.

inflammation. Leukotaxine does not exhibit the properties of histamine. The evidences supporting this fact have been described in detail elsewhere.[1, 2, 5, 6]

Bier and Rocha e Silva[7] have recently repeated the earlier studies of the writer on whole exudates and have confirmed the findings of a permeability and of a chemotactic factor in inflammatory exudates. They have, however, neglected to purify the exudates in order to obtain the relatively pure material capable *per se* of reproducing the increased capillary permeability and migration of polymorphonuclear leukocytes. On the basis of only meager evidence, they have concluded that the permeability factor (but not the chemotactic factor) is histamine. Their conclusions have been reached after extracting histamine from exudates, by the method of Barsoum and Gaddum[8] in order to eliminate thus the presence of any depressing substances when the end-product is tested on the isolated strip of guinea pig intestine. They admit, as I have pointed out previously,[1, 5] that with the accumulation of trypan blue there is a difference in the staining pattern of tissue as elicited by histamine and by an inflammatory exudate.† Yet these investigators assume, without any evidence, that this is presumably referable to the loose combination of histamine with proteins or by adsorption of colloids. In brief, the observations of these investigators on the contraction of the isolated intestine of the guinea pig simply indicates that histamine probably exists in exudates, and can by appropriate extraction, be demonstrated. This has been surmised and known (Loos[9]), as I have recently pointed out elsewhere.[2, 5, 6] In other words, the possibility that histamine exists in exudates has not been wholly dispelled by the available evidence on the presence of the permeability factor;[1, 2] but the primary importance of histamine in explaining the mechanism of increased capillary permeability in inflammation has been open to serious doubt.[1, 2, 5, 6] The permeability factor (*i. e.*, leukotaxine) recovered and isolated from an exudate displays, as pointed out elsewhere, none of the specific properties of histamine. Yet leukotaxine is capable *per se* of reproducing the same type of reaction as the whole exudate. The mutual, non-specific properties of

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[5] Menkin, V., *Physiol. Rev.*, 1938, **18**, 366.

[6] Menkin, V., and Kadish, M. A., *Am. J. Physiol.*, 1938, **124**, 524.

[7] Bier, O., and Rocha e Silva, M., *Arqu. d. Inst. Biologico*, 1938, **9**, 109, 123, 129.

[8] Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, **85**, 1.

† This is generally found to be the case even when the concentration of histamine is as low as 1:500,000.

[9] Loos, H. O., *Arch. f. Dermat. u. Syph.*, 1931, **164**, 199.

leukotaxine and histamine, (*e. g.,* dialyzing property, etc.) listed by Rocha e Silva and Bier, seem quite insignificant for the most part, inasmuch as they are true of numerous other unrelated substances. These investigators have failed to repeat the work on the chemical extraction of leukotaxine from exudate. They have worked solely with whole exudates. They have thus been unable to establish the fact that leukotaxine is histamine; whereas the writer has definitely demonstrated that leukotaxine has a great many physiological and chemical properties differing from those of histamine.[5, 6]

A single very important concrete evidence which nullifies the contention of these investigators is indicated by the following observation: A sample of leukotaxine, capable *per se* of actively inducing increased capillary permeability and leukocytic migration is extracted for the presence of histamine by the method of Barsoum and Gaddum.[8] According to the contention of the Brazilian workers,[7] by this procedure, the presence of any depressing impurities in leukotaxine, capable *per se* of overshadowing the contractile effect on the intestine of histamine would thus be eliminated. The final extracted material not only fails to produce a positive Zimmerman reaction for histamine; but it fails to induce an enhanced contraction of the isolated segment of guinea pig intestine (Fig. 1). Subsequent application of histamine to the same segment is followed by a powerful contraction, indicating thus the absence of any refractory effect[8] (Fig. 1). These additional observations on leukotaxine render it difficult even to consider the possibility of its identity with histamine.

Furthermore, the contention that hydrolysis with sodium hydrox-

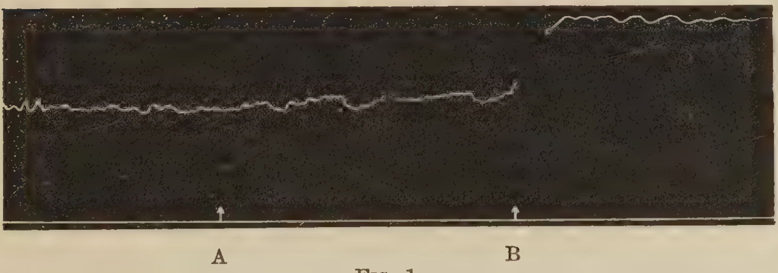

FIG. 1.

A—100 mg of an active fraction of leukotaxine extracted for histamine by the method of Barsoum and Gaddum.[8] The final product was added to a tyrode bath in which there was suspended an isolated strip of guinea pig intestine. Note the absence of any contractile effect.

B—After a few minutes, histamine dihydrochloride in a dilution of 1:5,000 was added to the tyrode bath the capacity of which was 20 cc. Note the immediate sharp contractile effect in contrast to the absence of effect with leukotaxine. It is also interesting to note the absence of any refractory effect. This indicates that leukotaxine evidently contains no histamine.[8]

ide dissociates the permeability factor from the chemotactic factor[7] is not substantiated when such a hydrolysis is carried on the purified active substance, leukotaxine. It is true that in earlier studies it was found that exposure to normal NaOH for several hours seemed to inactivate the partially purified material.[2] However, the claim of Rocha e Silva and Bier[7] of inactivating, in whole exudates, the permeability factor by alkaline hydrolysis without apparently affecting its chemotactic property is not necessarily an evidence for the presence of 2 different substances. An active group in a single substance responsible for one physiological property may well be inactivated without affecting other properties displayed by the particular substance. In order to establish whether alkaline hydrolysis is capable of dissociating the two biological properties of leukotaxine, the writer hydrolyzed for 16 to 22 hours samples of purified leukotaxine, in 1.1 N NaOH. This procedure failed completely to dissociate the two properties. The hydrolyzed product, when approximately neutralized immediately preceding its intracutaneous injection in rabbits, induced both the characteristic increased capillary permeability and leukocytic migration. Alkaline hydrolysis thus had failed to dissociate the two properties of leukotaxine.‡

Conclusions. The permeability and the chemotactic factors found in inflammatory exudates exist as well in the crystalline nitrogenous substance, leukotaxine, recovered from such exudates by chemical fractionation. There is no evidence to support the contention that the permeability factor is histamine. Extraction by the method of Barsoum and Gaddum has failed to reveal the presence of histamine in leukotaxine. Furthermore, alkaline hydrolysis of leukotaxine does not dissociate the permeability from the chemotactic factor. The foregoing observations add further support to the view that leukotaxine is the substance liberated from injured tissue which is primarily responsible for the basic sequences of the inflammatory reaction, namely, the initial increase in capillary permeability and the prompt migration of polymorphonuclear leukocytes.

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‡ In regard to the statement[7] that various products of protein metabolism, such as amino acids, are capable of inducing leukocytic migration, it is interesting to note that observations with a number of representative amino acids (as well as histamine) have failed to reveal leukocytic migration.

10322

Attempt to Produce Sarcomas in Rats from Ingestion of Crude Wheat Germ Oil* by Ether Extraction.

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Rowntree and his coworkers[1, 2, 3] recently reported the production of sarcomas in rats from the ingestion of a crude wheat germ oil made by extracting wheat germ according to Squibb's method. Ninety-three Wistar rats developed one or more intraäbdominal tumors. The time necessary for the production of these tumors was shortened by increasing the dose of the oil, either mixed with the diet or administered orally. Ten Wistar rats which received 1 cc of the crude wheat germ oil spread over the food, developed tumors within 200 days, and by increasing the dose to 4 cc administered in the same way, tumors were palpable as early as 15 days in some animals and in all within 111 days. Tumors were also produced in the Buffalo and Yale albino strains.

We undertook an experiment to determine whether a crude wheat germ oil extracted from fresh wheat germ by Squibb's method would induce sarcomas in rats. Since Rowntree furnished no specifications as to the kind of ether used, except that it was peroxide-free, we distilled our ether over solid sodium hydroxide to remove the peroxides. The crude oil was perfectly clear at first, but a large amount of sediment was deposited upon standing in the refrigerator. The oil was always thoroughly shaken before it was used. Rowntree's stock diet was used throughout the experiment and the rats were kept individually in screen bottomed cages.

Beginning September 9, 1937, 3 pairs of Wistar albinos and 3 pairs of Sprague Dawley albinos were given the crude wheat germ oil mixed with the diet, each animal receiving 1 cc daily. Similarly 1 cc of refined wheat germ oil was given to 2 pairs of both strains. The initial weight of the Wistar rats varied from 89 to 122 g, the

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* The author wishes to express his appreciation to Dr. R. C. Sherwood of General Mills for supplying the wheat germ and the refined wheat germ oil.

† Present address: The Barnard Free Skin and Cancer Hospital, St. Louis, Mo.

[1] Rowntree, L. G., Lansbury, John, and Steinberg, Arthur, Proc. Soc. Exp. Biol. and Med., 1937, **36**, 424.

[2] Dorrance, G. M., and Ciccone, E. F., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 427.

[3] Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

Sprague Dawley from 111 to 225 g. On April 6, 1938, after 209 days of feeding the oils, there were no palpable tumors; the dose of the oils was then increased to 3 cc per rat per day for 22 days. During this time 2 Sprague Dawley rats which had received the crude oil were found dead, but autopsy revealed no macroscopic tumors. Then for 27 days each of the remaining 10 rats received 3 cc of the crude oil daily by stomach tube. The refined oil was mixed with the diet as before. This forced feeding was followed by a drop in food consumption resulting in emaciation. At the end of this period both groups of rats were killed, but there was no evidence of tumors on autopsy.

The vitamin E content of the refined and crude wheat germ oils was approximately the same, 1.5 g of each sufficing for a successful gestation.

Conclusions. Tumors have not been observed in 20 albino rats of 2 strains, Wistar and Sprague Dawley, which were fed a crude and a refined wheat germ oil for 258 days. Perhaps all wheat germs do not contain the sarcogenic agent. Certain nutritional conditions may influence tumor production, and there may be subtle factors, as yet unappreciated, incidental to the preparation of the crude wheat germ oil.

10323

Hemoglobin-Methemoglobin and KCN.

MATILDA MOLDENHAUER BROOKS.*
From the University of California, Berkeley.

In a previous paper[1] it was found that the addition of comparatively small amounts of KCN to oxyhemoglobin in solution had no effect on the absorption maxima of the spectrum, as determined by spectrophotometric studies. The maxima for oxyhemoglobin solutions and for oxyhemoglobin containing KCN were identical. It was also found that when KCN was added to reduced hemoglobin, reduced by adding Stoke's reagent, the absorption spectrum again reverted to that of oxyhemoglobin. These facts were then interpreted to mean that KCN must form a compound with the bivalent form of hemoglobin. But since there was no change in color and no evolution of O_2, it was assumed that the change could not have occurred by the addition of the CN radical to the prosthetic group and it was suggested that perhaps some rearrangement took place in the uncolored portion of the hemoglobin compound.

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* I wish to express my thanks to Dr. Kubowitz for his assistance.

[1] Brooks, M. M., *Am. J. Physiol.*, 1935, **114**, 160; PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1228.

Further experiments which were done this summer at the Kaiser Wilhelm Institute with the kind coöperation of Dr. Otto Warburg have helped to clear up some of these questions.

The KCN-oxyhemoglobin absorption spectrum. For these experiments pure crystals of oxyhemoglobin freshly prepared from rat blood were used. The crystals were dissolved in either glycocoll buffer at pH 9.0 or phosphate buffer at pH 7.4 or 5.5. Dilutions were made from 10 to 1%.

The spectroscope used was a Schmidt and Haensch hand spectroscope with scale. No ratios between designated wave lengths could be measured and therefore the results are not quantitative as would be obtained by means of a spectrophotometer. Reagents were used in the same concentrations as described previously.[1]

The absorption spectrum of oxyhemoglobin solution alone was the usual one, with 2 maxima, the greater at 576 mμ and the lesser at 543 mμ.

The addition of an equivalent number of moles of KCN to oxyhemoglobin had no effect upon the absorption maxima. When the solution was reduced by bubbling in argon the absorption spectrum changed to that of reduced hemoglobin with the maximum at 555 mμ. This method was eminently satisfactory, while in the former experiments an attempt was made to reduce the defibrinated blood and cyanide mixture by vacuum. Because of the inadequacy of this procedure the experiment was unsuccessful.

When oxygen was again admitted to replace the argon, the spectrum changed again to that of oxyhemoglobin. These processes could be repeated. This experiment shows that KCN does not combine with the prosthetic group of the bivalent form of hemoglobin. This is in keeping with such old observations as those of Preyer,[2] v. Zeynek,[3] Kobert.[4]

The KCN-methemoglobin absorption spectrum. In one experiment (Table I) oxyhemoglobin was treated with K ferri CN or $NaNO_2$ to produce methemoglobin. In the former case the spectrum

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[2] Preyer, W., *Die Blusäure*, 1868.

[3] v. Zeynek, R., *Z. physiol. Chem.*, 1901, **33**, 426.

[4] Kobert, R., *Arch. f. Physiol.*, 1900, **82**, 603.

TABLE I.
Experiments with Oxyhemoglobin.

	Wave length of maximum absorption, in $m\mu$
A. Oxyhemoglobin alone	576, 543
Added K Ferri CN	543
” KCN to above	543
B. Oxyhemoglobin alone	576, 543
Added $NaNO_2$	576, 543, 620
C. Oxyhemoglobin alone	576, 543
Added KCN	576, 543

gave only the wave lengths at 543 $m\mu$ and the band at 576 $m\mu$ had disappeared, showing that hemoglobin was completely oxidized. In the latter case, when $NaNO_2$ was used in neutral or acid solution, both bands at 543 $m\mu$ and 576 $m\mu$ appeared. This showed that the $NaNO_2$ had not completely oxidized all of the hemoglobin and that some oxyhemoglobin was still present. The band at 620 $m\mu$ appears in acid or neutral solutions of methemoglobin but not in alkaline solutions.

TABLE II.
Experiments with Reduced Hemoglobin.

	Wave length of maximum absorption, in $m\mu$
A. Reduced hemoglobin in argon	555
Added KCN in argon	555
Replaced argon with O_2	576, 543
B. Reduced hemoglobin in argon	555
” ” + $NaNO_2$ in argon	620, 543
Added KCN in argon to above	Around 550

In another experiment (Table II) oxyhemoglobin was reduced by means of argon gas. The addition of KCN did not change the spectrum. But when argon was displaced by O_2 the spectrum changed to that of oxyhemoglobin with the usual 2 maxima at 576 $m\mu$ and 543 $m\mu$ again present. When $NaNO_2$ was added to reduced hemoglobin in the presence of argon, the spectrum of neutral methemoglobin was produced, with the maxima at 543 $m\mu$ and 620 $m\mu$. The addition of KCN to this (under an atmosphere of argon) gave a broad absorption band around 550 $m\mu$. The band at 620 $m\mu$ failed to appear because the solution had become alkaline. These results show that KCN combines only with the trivalent form of hemoglobin.

In a third experiment (Table III) the presence of methylene blue, when added to hemoglobin reduced by argon, produced no change in the absorption maximum. When oxygen displaced argon

TABLE III.
Experiments with Methylene Blue.

	Wave length of maximum absorption, in mμ
A. Reduced hemoglobin alone, in argon	555
” ” + methylene blue	No change
Shook in oxygen	576, 543
Added KCN in presence of O_2	No change
Replaced O_2 by argon	555
Replaced argon by O_2	576, 543
B. Methemoglobin + KCN + methylene blue in argon	543
Replaced argon by O_2	543

the spectrum of oxyhemoglobin appeared. When KCN was added to this no change took place. When argon again replaced oxygen, the spectrum of reduced hemoglobin appeared. This shows that methylene blue produces its effect only in the presence of oxygen. When no oxygen was available methylene blue did not oxidize hemoglobin. In the short time of the experiment no methemoglobin produced by the action of methylene blue on oxyhemoglobin in the absence of reductants was observable.

Summary and Conclusions. These experiments show that KCN combines only with the trivalent form of hemoglobin. Since it does not combine with the bivalent form, the presence of CN^- in the blood system of animals poisoned by CN^- is not the factor which produces death; its lethal effect is rather due to its action on the oxidizing enzymes in the tissues.

Two substances have been used as antidotes for cyanide poisoning, methylene blue and $NaNO_2$ The action of these two substances is different and can now be clarified as follows:

In the case of $NaNO_2$, methemoglobin is formed in the blood stream, cyanide combines readily with this trivalent form and is taken out of the system as the blood circulates forming cyanmethemoglobin. This of course inactivates a certain percentage of hemoglobin, but the enzymes of the tissues are freed and are again able to function.

In the case of methylene blue, no appreciable concentrations of methemoglobin are produced in the blood stream as the oxidation-reduction potentials of the combined system of methylene blue plus reductants in the blood are too low.[1] Therefore the dye produces its action by catalysis, activating oxygen and actual substitution in place of the poisoned enzymes transferring oxygen to the tissues until the enzymes are again able to function.

K ferri CN and $NaNO_2$ are strong oxidants compared with methylene blue. The ferro-ferricyanide system, according to Clark's scale[5] is far at the end of the scale near O_2 itself. $NaNO_2$ on addition to oxyhemoglobin still gives a weak band at 576 mμ, indicating that only a certain proportion of the oxyhemoglobin has been changed to methemoglobin. The methylene blue system is a weak oxidant located almost at neutrality in the pH-Eh oxidation-reduction scale.

10324 P

Growth of Small Numbers of Acid-fast Bacteria in Blood and in Serum.*

BROCKENBROUGH EVANS AND JOHN H. HANKS. (Introduced by Leland W. Parr.)

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, George Washington University, Washington, D. C.

The survival or growth of tubercle bacilli in blood is an important question from several points of view: the immunological action of serum and leucocytes, the investigation of tuberculous bacillemia, and the suitability of blood as a culture medium. Some investigators have emphasized the unfavorable action of blood on the growth of tubercle bacilli on solid media, while others have reported the growth of these organisms in blood. These points of view were reconciled when it was learned that the influence of blood in the two situations is quite different. This note indicates that, although blood interferes with the growth of tubercle bacilli on a solid substrate, it is actually an excellent medium.

Saline, defibrinated rabbit blood, or serum, .45 cc, were mixed with .05 cc volumes of 4 decimal dilutions of "clump-free" suspensions of human, bovine, avian, and timothy bacilli in 10 x 75 mm Pyrex tubes. Of these mixtures .05 cc was spread evenly on each of two slants of Corper's egg-yolk medium (in screw-capped tubes) which were free of surface moisture. The small tubes containing the mixtures were sealed with paraffined corks; both solid and liquid mediums were incubated with their surfaces horizontal.

From the results in Table I, several conclusions may be drawn: (a) Rabbit blood and serum were as favorable as the solid medium, with two exceptions: the irregular growth of bovine bacilli in blood,

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[5] Clark, W. M., *Studies on Oxidation-Reduction.*

* Aided by a grant from the U. S. Public Health Service.

TABLE I.

	Growth on Corper's medium inoculated with mixtures from:										Growth in rabbit serum and blood								
			bloods*									bloods*							
Culture	saline	serum pooled	1	2	3	4	5	6	7	8	serum pooled	1	2	3	4	5	6	7	8
H-37	++	++	4	9	1	3	1	7	3		+	+	+	+	+	+	+	+	
(human)	21	30	—	1	2	—	—	—	—		+	+	+	+	+	+	+	+	
	1	—	—	—	—	—	—	—	—		+	+	+	+	+	+	+	+	
R.S.	31	40	—	—	1	2	—	1	—	3	+	+	+	+	+	+	+	+	+
(bovine)	9	11	—	—	—	—	—	2	—	—	+	+	+	+	+	+	—	+	—
	1	—	—	—	—	—	—	—	—	—	+	—	+	+	—	+	—	—	—
A-12	++	++	16	40	16	24	38	29	40	12	+	+	+	+	+	+	+	+	+
(avian)	23	18	—	—	3	—	3	1	2	2	+	+	+	+	+	+	+	+	+
	—	2	—	—	—	—	—	—	—	—	+	+	—	—	+	+	—	—	+
No. 581	++	++	++	++							+	+	+						
(timothy)	13	18	3	9							+	+	+						
	9	1	—	5							—	+	+						

*Each small column represents blood from a different rabbit. The results in solid medium show colony-counts from 0.1 cc (2 tubes); ++ means more than 50 colonies. In liquid media, + means growth, checked by staining. Only the 3 highest dilutions could be shown. The growth of timothy bacilli in rabbit serum was delayed.

and the delayed growth of timothy bacilli in serum. (b) There was no evidence of the growth-inhibitory effects hitherto ascribed to hemoglobin or to "autolytic products" of blood. (c) The failure of tubercle bacilli to grow suitably on solid medium inoculated with blood was again confirmed. Positive results cannot be obtained with small numbers of bacilli.

Since these results were confirmed by quantitative studies on human tubercle bacilli in human blood, it seems that the surest way to demonstrate bacilli in human blood is by the incubation of the blood.

Growth of small numbers of bacilli was obtained also in liquid substrates composed of: serum-leucocyte mixtures, blood or serum diluted with water or with Long's medium, in polymorphonuclear leucocytes and saline, and in the washed cellular fraction of blood suspended in saline or dilute Long's medium. The more dilute systems appear equally favorable for growth and offer the advantages of economy of materials and greater ease in seeing the growth.

10325

Intra-Arterial Hypertonic Saline Solution in Experimental Shock.

DOUGLAS B. KENDRICK, JR., AND KHALIL G. WAKIM. (Introduced by Hiram E. Essex.)

From the Institute of Experimental Medicine, The Mayo Foundation, Rochester, Minn.

Davis and his coworkers[1, 2] have reported prolonged elevation of blood pressure after intra-arterial administration of 5 to 10 cc of 25% solution of sodium chloride. The pressor effect of such injections was thought by these workers to be predominantly nervous in origin, since it disappeared after section of the spinal cord. The possibility of using hypertonic solutions of sodium chloride as an emergency treatment of traumatic shock was suggested. The experiments reported in this paper were done to determine the efficacy of such treatment.

The experiments were carried out on dogs anesthetized by means of nembutal (pentobarbital sodium), given intravenously in doses

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[1] Davis, H. A., *M. Ann. District of Columbia,* 1937, **6**, 344.

[2] Davis, H. A., Jermstad, R. J., and Choisser, R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 144.

of 25 mg for each kilo of body weight. Observations were made on normal anesthetized dogs, on anesthetized dogs in a state of shock produced by intestinal manipulation and on dogs whose spinal cords had been sectioned at the level of the seventh cervical vertebra. Observations were also made on dogs following adrenalectomy.

In both normal animals and those in a state of shock, 5 to 10 cc of a 25% solution of sodium chloride injected intra-arterially gave a vasopressor effect. In a few experiments the blood pressure was elevated from 60 or 70 mm of mercury to over 100 mm of mercury. Given intravenously, the same doses of hypertonic saline solution produced vasodepressor responses both in the normal animal and in animals in a state of shock. Whether the injections of hypertonic saline solution were given intra-arterially or intravenously the animals appeared to be harmed by their use.

Following section of the spinal cord, injections of 5 to 10 cc of 25% saline solution given intra-arterially gave a small but transient rise in blood pressure. Intravenous injections gave a slight elevation of blood pressure, which was followed by a transient fall.

In bilaterally adrenalectomized animals that were otherwise intact, the same doses of concentrated saline solution given intra-arterially caused a small transient rise in blood pressure. After shock had been produced in these animals, the same dose of hypertonic saline solution given intra-arterially produced little if any effect on the blood pressure. Intravenous injections into these animals produced a sudden, marked fall in the blood pressure, which soon returned to the former level and then rapidly dropped to a critical level.

In a single trained animal an intra-arterial injection of 25% solution of sodium chloride resulted in such untoward effects that further injections were considered inadvisable. Even under surgical anesthesia intra-arterial administration of a concentrated solution of sodium chloride resulted in marked spasms and contractions of the skeletal muscles and drastic purgative and diuretic effects.

It was found that the blood pressure of animals that had received 5 to 10 cc of 25% solution of sodium chloride was rapidly lowered by subsequent slight intestinal manipulation. Furthermore, the blood pressure of dogs in shock could not be raised by a second injection when the effect of the first injection had been dissipated. It was a consistent finding that the blood pressure of animals in shock gradually declined to critical levels. Death appeared to be hastened rather than delayed by the injections.

Davis and his coworkers attributed the vasopressor effects of intra-arterial injections of 25% solution of sodium chloride to "stimulation of the peripheral nerve endings in the involved area with a resultant generalized vasoconstriction affected by the central nervous system."

Conclusions. The findings of Davis and coworkers on the effect of administering a concentrated solution of sodium chloride have been corroborated. However, it seems apparent from our observations that the intra-arterial administration of 25% saline solution cannot be recommended as an emergency treatment for secondary shock. In spite of the immediate vasopressor response and in spite of the fact that the increased blood pressure may be maintained for a while, the final result of the injections is severe injury to the recipient.

10326 P

Technic for the Study of Secretions from the Oral Mucosa in Humans.

A. L. WINSOR AND B. KORCHIN. (Introduced by J. A. Dye.)
From Cornell University.

To our knowledge the secretion from the oral mucosa of humans has been observed[1, 2] but has neither been collected nor measured. Shafer[3] wrote that, "Secretion of the mucus glands of the mouth have not been obtained in man." However, Montgomery and Stuart[4] found that secretions from the oral mucus membrane can be directly observed in dogs and humans. They reported that, "Like the salivary glands they do not secrete continuously, but only in response to a large number of unconditioned and conditioned reflex stimuli." But the repeatedly confirmed findings of a continuous parotid gland flow in humans in the absence of marked exterostimulation and the fact that the oral mucosa and the parotid glands have a similar type of neural innervation suggested the possibility that more accurate measures would be more revealing. We were able to collect and measure both inactivated as well as activated secretion from the oral mucosa of human subjects by means of the following technic:

Small discs similar to those used for the collection of parotid secretion[5] are fastened by suction over different regions of the oral

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[1] Cerutti, P., and Verzola, M., *Gior. ital. di dermate. esif.*, 1935, **76**, 1269.

[2] Montgomery, M. F., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 717.

[3] Shafer, E. A., *Textbook of Physiology*, 1898, Vol. 1, p. 344.

[4] Montgomery, M. F., and Stuart, J. S., *Am. J. Physiol.*, 1936, **115**, 497.

[5] Lashley, K. S., *J. Exp. Psychol.*, 1916, **1**, 461.

mucosa. The discs consist of 2 separate chambers. Both chambers are supplied with slender rubber tubes which pass out through the mouth. The disc is held in place by means of a slight amount of suction which can be applied by gently sucking the exposed end of the rubber tubing which is attached to the outer chamber. The secretion is free to flow through the inner chamber and push a bead of water through a calibrated capillary tube which is attached to the rubber tubing that leads out from the inner chamber. The secretory pressure of the inactivated as well as the activated secretion is adequate to move the bead of water through the capillary tubing.

The secretion from the oral mucosa is the most viscous of the salivary secretions. If the discs are removed with care the secretion adheres to the discs and may be collected for qualitative analyses. In some cases an hour may be necessary before a 0.05 cc sample of inactivated secretion can be obtained. Activated or stimulated secretions can be obtained more readily. The secretagogic activity of chewing dry bread serves adequately when larger samples are desired. In our studies on parotid secretion[6] we found the parotid secretory activity to be a sensitive index of organic changes. Although the glands in the oral mucosa have a similar neural innervation and are readily accessible, their excessive viscosity and slow rate of flow limit their use for this purpose. However, by means of the suction discs it is possible to collect samples of secretion which may be of some diagnostic aid.

These discs, which were originally developed by Lashley can also be used in some cases as a substitute for cannulation in the study of other glandular activities in animals.

10327

Rôle of Capsule in Suprarenal Regeneration Studied With Aid of Colchicine.

D. D. Baker and R. N. Baillif. (Introduced by R. Ashman.)

From the Department of Anatomy, School of Medicine, Louisiana State University, New Orleans.

All the successful functional transplants reported in the literature seem to depend upon the creation of an initial suprarenal insufficiency, as originally postulated by Halsted.[1] Such studies have been made by Jaffe and Plavska,[2] Blodinger, Klebanoff and Laurens,[3] Higgins and Ingle,[4] Wyman and tum Suden,[5] Ingle and Higgins,[6] and Baker and Baillif.[7]

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[6] Winsor, A. L., and Korchin, B., *J. Exp. Psychol.*, 1938, **23**, 62.

[1] Halsted, *J. Exp. Med.*, 1909, **11**, 175.

The study herewith reported is an endeavor to locate the sites from which regeneration takes place in the enucleated suprarenal gland, and to follow the process of regeneration from the time of transplantation until the completion of the regenerative process. A recent study along somewhat the same lines by Zwemer, Wotton, and Norkus[8] also concerns itself with the histology of the normal adrenal cortex and the origin of new and removal of old corticoadrenal cells under experimental conditions.

Suprarenal insufficiency was produced in 37 immature and mature male and female albino rats by the removal of the right gland and the enucleation of the left, according to the procedure devised by Ingle and Higgins.[6] In a few instances the capsule and residual cortex were transplanted in the abdominal wall. All animals survived the operation.

The usual laboratory diet was employed, and no cortin or sodium chloride was added to it. The animals were sacrificed at daily intervals from the first to the tenth day, and at weekly intervals thereafter to the fifty-seventh day.

Because the site of most active cell proliferation during the process of regeneration is marked by numerous mitoses, we endeavored to make this phenomenon more evident by the use of colchicine, which, with a few exceptions, was given subcutaneously 9.5 hr before the animals were sacrificed.

The tissue to be examined was fixed in Bouin's fluid and serially sectioned. The glands were stained with Heidenhain's azan mixture and Krichesky's modification of Mallory's triple connective tissue stain.

Within the first 3 postoperative days the capsule becomes edematous, hyperemic and thickened. By the 2nd day it has lost its dense, heavily-staining appearance and becomes more loosely constructed. The cells and their nuclei, especially in the inner half of the capsule, become oval, and many begin to undergo mitosis. Prominent blood vessels appear among these transforming cells.

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[2] Jaffe and Plavska, PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 528.

[3] Blodinger, Klebanoff and Laurens, PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 22.

[4] Higgins and Ingle, *Proc. Staff Meet. Mayo Clinic,* 1937, **12**, 69.

[5] Wyman and tum Suden, *Endocrinology,* 1937, **21**, 523.

[6] Ingle and Higgins, *Endocrinology,* 1938, **22**, 458.

[7] Baker and Baillif, *Anat. Rec. Suppl.,* 1938, **70**, 5.

[8] Zwemer, Wotton and Norkus, *Anat. Rec.,* 1938, **72**, 249.

The changes described in the capsular cells (Fig. 1) tend to produce a stratified appearance in the capsule as regeneration progresses. The innermost sheets of fibers become broken up, due to a centripetal migration of the former capsular cells. The process is probably not one of active movement but rather a change in the mitotic planes of the ingrowing cells. In early capsular hyperplasia the plane of mitosis is parallel to the inner surface of the capsule. As the process proceeds, the plane changes through an angle of 90° and the multiplying cells, as a result, begin to proliferate inward from the capsule. Later they fill the space left by removal of the inner portion of the gland. The cortex is thus replaced by cells derived from the substance of the capsule.

The newly formed cortical cell has a relatively large nucleus and a very small amount of cytoplasm. The nucleus is at first slightly fusiform, but very soon becomes oval. Then it increases rapidly in size and becomes round as the new cell pushes its way into the glomerular zone. The quantity of cytoplasm increases slowly.

The cells within the capsule which are being transformed into cortical cells begin to show a small amount of lipoid in their cytoplasm. The lipoid appears first as tiny droplets at one side of the nucleus, in the region of the centrosphere. As they increase in size, the droplets spread throughout the cytoplasm. In the course of cytological transformation the early cortical cell increases markedly in size, becomes round, and accumulates more and more lipoid. These changes are usually more marked after the cells pass the inner margin of the capsule, but may be seen while they are still enclosed by capsular fibers. Under these conditions an island of cortical cells arises within the capsule. The most active stage of cortical regeneration occurs between the second and seventh days.

Concomitant with the ingrowth of transforming cortical cells, a second cytological element is seen to proliferate from the capsule in the form of a small, stellate cell, which is apparently actively migrating inward. This cell appears after the process of cortical hyperplasia has begun, but soon outstrips the glandular cells in migration. It very rarely shows any stainable lipoid in its cytoplasm. This element is destined to supply the connective tissue support for the ingrowing glandular cells and blood vessels.

The cortical zones are seen to regenerate in the following manner:

a. Narrow cords of cells appear as ingrowths from the capsule, giving rise, by further ingrowth and multiplication, to the cell groups which are destined to become the zona glomerulosa.

b. From the inner margin of these cell groups, directed by the radially arranged connective tissue strands, new cell cords proliferate toward the center of the gland to form the zona fasciculata.

c. The inner ends of these cords show branchings among the connective tissue in the center of the gland, and a zona reticularis is seen after 3 weeks.

The tissue left *in situ* after enucleation of the gland is a mere shell

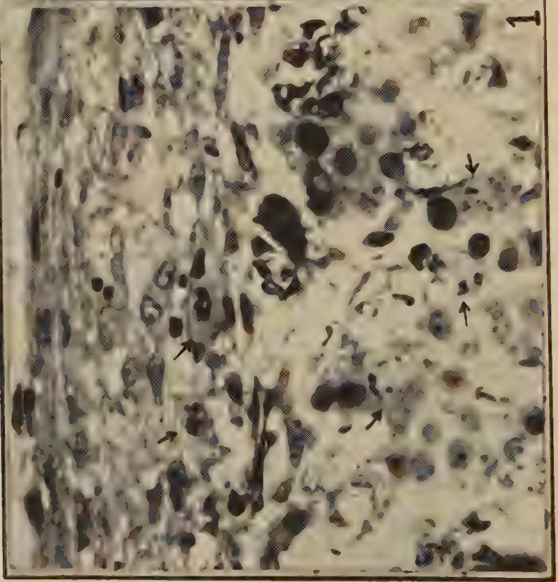

FIG. 1.
Mitotic cells are more frequently seen in the inner than in the outer half of the capsule. Colchicine. × 525. Some mitoses indicated by arrows.

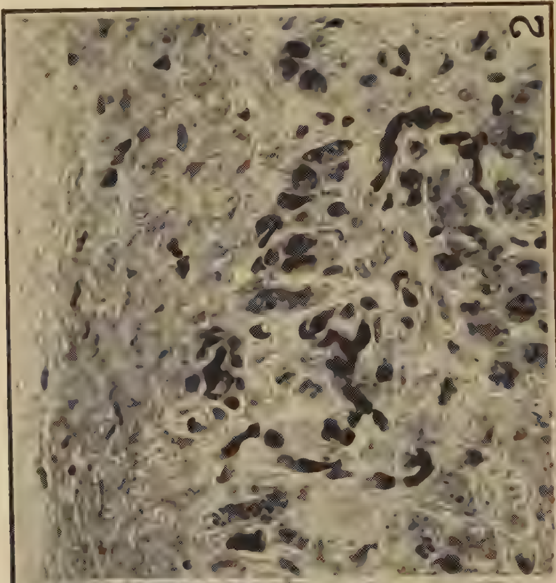

FIG. 2.
Few mitotic cells are seen without the use of colchicine. × 525.

which promptly becomes edematous and filled with a blood clot. This clot becomes organized rapidly and is canalized by ingrowth of the capsular connective tissue and blood vessels. This process is followed by a gradual resorption of the clot, beginning at the periphery, which allows space for ingrowth of the regenerating cortical cells.

Small but definitely marked islands of medullary tissue were noted in 4 regenerating glands removed after the 20th day. In 3 of these the islands are seen embedded in the edge of the central fibrous tissue mass, and in the 4th several small medullary islands are seen in the scar of the operative incision. These cells are readily distinguished from the adjacent cortical cells by their arrangement into pseudofollicular groups separated by a connective tissue stroma. It was also noted that they have a much more lightly staining cytoplasm than is characteristic of the cortical cells. It is quite possible that these are medullary cells which remained attached to nerve bundles and were not removed when the gland was enucleated, for nerve trunks can be traced to them through the capsule and cortex.

The actual process of regeneration of the suprarenal cortex seems to be practically completed within 30 days. Since no signs of suprarenal deficiency were noted in any of the animals, it may be assumed that the enucleated gland begins to function within a few days after operation. No operative deaths occurred, but younger animals seemed to withstand the procedure better than fully matured ones. Our observations reveal no noticeable difference between the sexes in the rate of regeneration.

Mitotic cells were much more numerous in the regenerating glands of animals receiving colchicine (Fig. 1) than in those that did not (Fig. 2). The ratio of mitotic cells before and after giving colchicine was 1 :4.6.

Summary. Cortical cells and their supporting tissues regenerate from the capsule of the enucleated suprarenal gland. Mitotic cells are more frequently seen in the inner half of the capsule. These new cells become oval and migrate toward the center of the regenerating gland, some to form cortical cells and others to form supportive tissue for the suprarenal cords. The cytoplasm of the new cortical cells very early contains lipoid, but that of the supportive cells contains no fatty substances. The central blood clot becomes organized and canalized.

In 4 glands nerves could be traced through the capsule to groups of medullary cells. The ratio of mitotic cells before and after the administration of colchicine was 1 :4.6.

10328

Effect of Some Short-acting Barbituric Acid Derivatives on Intestinal Activity *in vivo*.*

C. L. Burstein. (Introduced by Co Tui.)

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Controversial reports on the effect of barbituric acid derivatives upon intestinal activity have prompted the repetition of this study in an endeavor to obtain an explanation for the conflicting results. On the one hand, Gruber,[1, 2] using dogs with Thiry-Vella loops of the duodenum and jejunum, concludes that the barbiturates studied (sodium amytal, ortal sodium, and sodium phenobarbital) cause a fall in general tonus and in the amplitude of rhythmic intestinal contractions. On the other hand, Dreyer and Hebb,[3] showed that in decerebrate cats all the barbituric acid derivatives used by them (amytal, phenobarbital, dial, phanodorn, and pentobarbital) caused stimulation of gastric and intestinal movements.

The dogs used in this research were the same as those employed in earlier investigations.[4, 5] They consisted of 8 dogs prepared with Thiry-Vella loops of the upper jejunum. Tracings of intestinal movements were recorded after inserting a balloon, connected to a water manometer at 12 to 15 cm pressure, into the proximal end of the Thiry-Vella loop. Respiratory tracings by means of a pneumograph applied about the chest were simultaneously recorded in order to rule out the possibility that the tracings taken from the balloon were due to transmitted respiratory movements. These intercostal respiratory tracings also served to show the depth of anesthesia. No preanesthetic medication was administered. The barbituric acid derivatives used consisted of those most commonly employed for intravenous anesthesia: sodium N-methyl-cyclohexanyl barbiturate (evipal), sodium iso-amyl ethyl barbiturate (amytal), sodium iso-amyl ethyl thio-barbiturate (sodium thio-ethamyl), sodium

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*Grateful acknowledgment is expressed to Dr. W. F. Ruggiero for his aid in the surgical preparation of the Thiry-Vella Fistulas.

[1] Gruber, C. M., *J. Pharm. and Exp. Therap.*, 1936, **56**, 432.

[2] Gruber, C. M., Crawford, W. M., Greene, W. W., and Drayer, C. S., *J. Pharm. and Exp. Therap.*, 1931, **42**, 27.

[3] Dreyer, N. B., and Hebb, C. O., *Proc. Nova Scotian Inst. Sci.*, 1933-1934, **18**, 282.

[4] Burstein, C. L., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 530.

[5] Burstein, C. L., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 396.

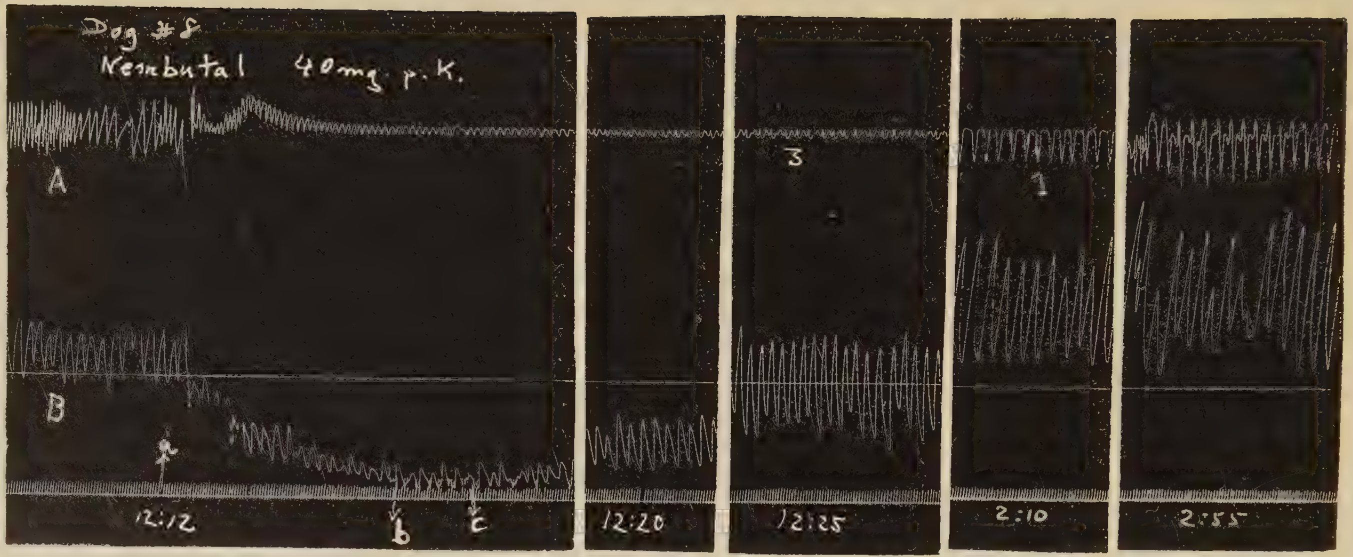

FIG. 1.

Effect of nembutal on intestinal activity of dog *in vivo*. Serial sections taken from the same experiment: Upper record (A), thoracic respirations; lower record (B), intestinal contractions. Time in second intervals. The numbers below the respiratory tracing indicate the plane of surgical anesthesia. Nembutal, 5% solution, 40 mg per kg body weight, was injected between (a) and (b). At (c) an endotracheal tube was inserted by the oral route.

N-ethyl-l-methyl butyl barbiturate (nembutal), and sodium N-ethyl-l-methyl butyl thiobarbiturate (pentothal). In each case, a 5% solution was freshly prepared and injected slowly intravenously until the lid reflex disappeared. The doses varied between 30 and 60 mg per kilo body weight. An endotracheal tube was introduced by the oral route as soon as surgical anesthesia supervened in order to assure a patent airway and obviate the effect of the ensuing anoxemia.

In every instance and with all the different barbiturates used the immediate effect, lasting from 5 to 15 minutes following the injection, consisted of a marked reduction in the amplitude of intestinal contractions and a fall in general tonus. Immediately following this primary depressing effect, however, there was a gradual rise in general tonus to above normal and increased rhythmic intestinal contractions which were maintained until the animal awakened. This secondary period during which anesthesia was still evident lasted from 10 minutes to 2 hours depending upon the rapidity of action of the particular barbituric acid derivative used. Fig. 1 shows the typical picture obtained with nembutal.

Conclusion. The short-acting barbituric acid derivatives studied when injected intravenously cause an immediate depression of rhythmic intestinal contractions and tonus. This primary effect, however, is transient and is succeeded by a more prolonged phase characterized by increase in both intestinal contractions and tonus.

10329

A Hematopoietic Substance in Liver.

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In two previous communications[1] the authors have shown that liver yields a peptide in some respects resembling a typical albumose capable of causing blood regeneration in pernicious anemia. Clinically active preparations were obtained which were completely free from pentose or other carbohydrate, purines, phosphoric acid, and sulphur. Other workers have stressed the presence of some or all of these groups or have postulated the accessory action of a variety of other chemical types which are absent from our material. Karrer,

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[1] Dakin, H. D., and West, R., *J. Biol. Chem.*, 1935, **109**, 489; 1936, **115**, 771.

Frei and Fritzsche,[2] without reference to our previous work, stated that the clinical activity of their material was proportional to the phosphoric acid content and that adenine and pentose were also present. More recently, they describe the active substance as free from these substances and regard it as a biuret negative peptide with an elementary composition closely resembling that of our product. No experimental data are furnished but some amino acids contained in our product are stated to be absent, while other amino acids of unspecified type are said to be present. We have recently assured ourselves of the absence of a nicotinic acid group in our peptide.

The following note consists of the results of a few experiments on the precipitation of our substance with typical albumose precipitants including nucleic acid, bile, taurocholic, and other bile acids and the barium carbamate reaction of Seigfried. We find that the first 3 reagents yield precipitates containing much potent material. Glycocholic acid and the carbamate reaction gave unsatisfactory results.

The precipitation with nucleic acid was carried out as follows: Thymonucleic acid (4 g) was dissolved in decinormal sodium hydroxide (50 cc), diluted to 200 cc, and acidified with glacial acetic acid (2 cc). This was added with stirring to 50 cc of a 10% solution of our peptide. An immediate precipitation occurred, and the mixture was placed in the refrigerator overnight. The heavy precipitate was washed with water, acetone, and alcohol. Approximately half of the peptide was precipitated. Dilute hydrochloric acid decomposed the precipitate only incompletely, but it was easily soluble in disodium hydrogen phosphate solution and was tested clinically in this form. (Cases 552,986 and 553,894.) Thirty milligrams gave a reticulocyte increase from 1.8 to 24.2%, and the R.B.C. count rose from 1.5 to 2.9 million. Twenty milligrams gave a good positive but submaximal response. The precipitation by nucleic acid of some but not all the albumoses in Witte's peptone was observed by Löbisch[3] many years ago.

Filtered bile was adjusted to pH 3.5 by addition of dilute sulphuric acid and added by degrees to a solution of the peptide (1 g) in water (10 cc). About 20 cc bile was needed for maximal precipitation. After washing the oily precipitate with water it was dissolved in 60% alcohol (5 cc), made alkaline with alcoholic ammonia, and the clear solution precipitated with absolute alcohol (25 cc). A significant amount of bile pigment was retained by the precipitate which was dissolved in water and tested clinically (Case 540,130). About 60% of the peptide was precipitated by the bile and its potency appeared to be slightly less than that of the original material.

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[2] Karrer, P., Frei, R., and Fritzsche, H., *Helv. chim. Acta*, 1937, **20**, 622; 1938, **21**, 314.

[3] Löbisch, W., *Beitr. z. chem. Phys. u. Path.*, 1906, **8**, 205.

Sodium taurocholate was dissolved in 10 parts of water and brought to pH 3.5 with dilute hydrochloric acid. The peptide (10 g) was dissolved in 20 parts of 3% acetic acid and the taurocholic acid added as long as precipitation occurred. The amount of taurocholic acid necessary was about one-third the weight of peptide. The finely divided precipitate was gently warmed to coagulate it and then after cooling, filtered off and washed with water. To regenerate the peptide, the precipitate was dissolved in a minimum of 60% alcohol, pyridine added (3 cc) to combine with the taurocholic acid, and then a large excess of acetone added. The recovered peptide only represented about 20% of the starting material. The precipitation of the peptide with taurocholic acid is apparently a reversible reaction between the 2 colloids. The very complete precipitation of proteins by taurocholic acid in contrast to its failure to precipitate the simpler peptones was observed by Maly and Emich[4] more than 50 years ago. On testing the material precipitated by taurocholic acid and that left in the filtrate it was found that both chemically and clinically there was no significant difference. (Cases 539,788, 543,019, 546,989, 481,608, 550,302.)

Summary. It has been shown that bile, taurocholic acid, and nucleic acid precipitate liver material active in pernicious anemia from aqueous solution.

10330 P

Changes in Human Blood Preserved for Transfusion.

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The purpose of the present studies was to determine the rate and mode of disintegration of human blood in various preservatives suitable for transfusion.

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[4] Maly, R., and Emich, F., *Monatschrift f. Chemie*, 1883, **4**, 89.

TABLE I.
Changes in Human Blood Stored at 2-5°C. (Values are for plasma after cells have been centrifugated out at end of period of storage.)

		Days Storage	Hemoglobin, g%	Potassium, g%	Glucose, g%	pH	Specific Conductance, mho
Exp. 1 (aerobic)		0	<.001	.011	2.36	7.74	.00977
blood	10 parts	5	.005	.037	2.32	7.75	.01044
5.4% glucose	13 ”	10	.011	.054	2.33	7.37	.01025
3.2% sodium citrate	2 ”	15	.024	.063	2.29	7.39	.01054
		20	.031	.075	2.29	7.55	.01040
		25	.088	.077	2.23	—	.01058
		30	.071	.078	2.18	7.35	.01017
		35	.087	.079	—	7.28	—
Exp. 2 (aerobic)		0	.008	.016	3.39	7.70	.00583
blood	10 parts	5	.015	.035	3.34	7.59	.00582
5.4% glucose	15 ”	10	.019	.050	3.15	7.71	.00623
heparin 3 mg/ 5 cc blood		15	.034	.060	2.97	7.36	.00627
		20	.058	.063	3.11	7.39	.00628
		25	.052	.065	3.14	7.47	.00641
		30	.138	.081	3.06	7.39	.00650
Exp. 3 (anaerobic)		0	<.001	.013	2.70	7.08	.00708
blood	10 parts	5	.003	.055	2.66	7.44	.00770
5.4% glucose	13 ”	10	.005	.064	2.33	7.46	.00781
3.2% sodium citrate	2 ”	15	.006	.066	2.53	7.28	.00774
		20	.007	.065	—	7.69	.00839
		25	.010	.096	—	7.78	—
		33	.016	.077	—	7.18	.00793
			*	*			
Exp. 4 (aerobic)		0	<.001	.012	.62	7.81	.01364
blood	21 parts	5	.051	.107	.61	8.08	.01413
5.4% glucose	2 ”	10	.247	.137	.56	7.84	.01382
3.2% sodium citrate	2 ”	15	1.013	.154	.51	7.73	.01300
		20	1.690	.162	.49	7.62	.01241
		25	2.370	.161	.47	7.46	.01187
		30	2.380	.167	.49	7.29	.01303
Exp. 5 (aerobic)		0	<.001	.012	.03	7.84	.01804
blood	10 parts	5	.012	.069	.01	7.32	.01830
0.95% NaCl	13 ”	10	.112	.087	.00	7.69	.01854
3.2% sodium citrate	2 ”	15	.381	.097	.00	7.58	.01778
			†	†			
Exp. 6 (aerobic)		0	<.001	.016	.07	7.93	.01478
blood	23 parts	5	.026	.153	.02	8.03	.01609
3.2% sodium citrate	2 ”	10	.214	.179	.01	7.88	.01557
		15	.524	.193	.00	7.64	.01477

*To compare with values in Experiment 1 divide figures in these columns by 2.1.
†To compare with values in Experiment 1 divide figures in these columns by 2.3.

Blood was drawn from healthy adults in amounts of 300 to 600 cc in 1500 cc Erlenmeyer flasks containing the preservatives to be studied. The blood mixtures were then apportioned aseptically, 50 cc into each 250 cc Erlenmeyer flask, plugged with sterile cotton and stored at 2-5°C. Evaporation was nil. Bacterial contamination was excluded. At 5-day intervals one flask of the series was withdrawn from storage, the cells and plasma thoroughly mixed by careful shaking, and the entire sample centrifugated. The plasma was then pipetted off for analysis.

The preservatives used were: Anhydrous dextrose, U.S.P. (Merck), and anhydrous dextrose (Eastman Kodak Co.). Sodium citrate ($Na_3C_6H_5O_7 + 2H_2O$) (Merck's Reagent Grade). Heparin (Connaught Laboratories).

Plasma hemoglobin was determined by the method of Wu,[1] plasma potassium by the technic of Truszkowski and Zwemer,[2] plasma glucose by the procedure of Shaffer, Hartman, and Somogyi,[3] pH of plasma by the Coleman glass electrode, and specific conductance of plasma by the standard methods.[4]

The Rous-Turner preservative[5] was modified by changing the proportions of blood, glucose, and citrate from 30:50:20 to 40:52:8, thus reducing the concentration of sodium citrate from 0.76% to 0.256%.

Table I presents data obtained from representative experiments. In no blood mixture was there total absence of hemolysis after 5 days' storage. The rates of hemolysis were definitely slower when large amounts of glucose were present. The K ions migrated into the plasma at the same rate in all preservatives; this process was independent of the amount of Hb in the plasma. The migration of K ions was not dependent upon the concentration of Na in the plasma (see No. 5) nor upon the presence of citrate ion (see No. 2). The pH changed from 8.00 to 7.00. Glycolysis proceeded at the temperature of storage for approximately 15 days.

Experiment 3 demonstrates the remarkable inhibition of hemolysis under anaerobic conditions (compare with No. 1), even though the K migration proceeds at the same rate as in blood exposed to air. We have employed the mixture in No. 1 for 35 transfusions without untoward reaction.

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[1] Wu, H., *J. Biochem.*, 1922, **2**, 189.

[2] Truszkowski, R., and Zwemer, R. L., *Biochem. J.*, 1937, **31**, 229.

[3] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

[4] Hawk and Bergeim, *Practical Physiological Chemistry*, 11th Edition, P. Blakiston's Son & Co., Philadelphia, pp. 42-45.

[5] Rous, Peyton, and Turner, J. R., *J. Exp. Med.*, 1916, **23**, 219.

10331 P

Influence of Fever Therapy on Blood Levels and Urinary Excretion of Ascorbic Acid.

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There is more or less agreement that the normal ascorbic acid content of the blood ranges between 0.7 to 2.0 mg %. Mirsky[1] reports a range of 1.19 to 2.66, and Taylor and Faulkner,[2] 1.70 to 2.24. Low values, .14 to 1.21, have been found in various pathological states by Gabbe,[3] and Taylor and Faulkner[2] reported .11 to .55 in cases with clinical manifestations of scurvy. In general there seems to be very little agreement between the pathological state and the blood ascorbic acid except in cases of infection with elevated temperature where the values are consistently low. (Martin and Heise,[4] Harris, *et al.*,[5] and Abbasy, *et al.*[6])

Moreover, the blood level seems to be no indication of the body's need, depletion or saturation, and must always be studied in connection with the intake and the urinary output.

Urinary excretion levels at the saturation point vary between 15 and 35 mg daily. (Stephens and Hawley,[7] 15-28 mg; Harris, Ward and Ray,[8] 30-35; and Ahmad,[9] 23-25 mg.) If a single large dose of 500 mg is given at this stage, 30-40% of this will be excreted in 24 hours. Total daily excretion may be much under the 15-35 mg during depletion or in pathological cases, and the percent excretion of a single test might likewise be much reduced. Blood levels in both situations may be well within the normal range.

During infection with elevated temperature, urinary excretion as well as blood levels is decreased. Abbasy, Hill and Harris[6] suggest that this indicates an unusual demand on the part of the body or a destruction in the body due possibly to the infecting organism.

This study followed the blood levels and urinary excretion of ascorbic acid in a group of patients receiving fever therapy electrically induced. In only one case studied was the infection active enough to elevate the temperature. The cases were, for the most part, neurosyphilitics and arthritics.

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[1] Mirsky, A., Swadesh, S., and Soskin, S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1130.

[2] Taylor, F. H. L., Chase, D., and Faulkner, J. M., *Biochem. J.*, 1936, **30**, 1119.

[3] Gabbe, E., *Klin. Wschr.*, 1934, **13**, 1389.

[4] Martin, G. J., and Heise, F. H., *Am. J. Digest. Dis. and Nutrition*, 1937, **4**, 368.

[5] Harris, L. J., Abbasy, M. A., Yudkin, J., and Kelly, S., *Lancet*, 1936, **1**, 1488.

[6] Abbasy, M. A., Hill, N., Gray, and Harris, L. J., *Lancet*, 1936, **2**, 1413.

[7] Stephens, D. J., and Hawley, E. E., *J. Biol. Chem.*, 1936, **115**, 653.

[8] Harris, L. J., Ray, S. N., and Ward, A., *Biochem. J.*, 1933, **27**, 2011.

[9] Ahmad, B., *Biochem. J.*, 1936, **30**, 11.

Blood vitamin C was determined by the Farmer and Abt[10] method which is microtitration with 2-6 dichlorophenolindophenol. Urinary ascorbic acid was determined on the 24-hour specimens according to the method of Harris and Ray,[11] which is titration with the same dye.

TABLE I.

Blood Levels and Urinary Excretion of Ascorbic Acid During Fever Therapy.

	Blood Ascorbic Acid			Urinary Excretion Ascorbic Acid	
Case	Before mg%	After mg%	Decrease mg%	During fever mg	48 hr later mg
E.D.	.7	.3	.4	10	13
	.6	.2	.4	8	13
	.6	.3	.3	8	18
	1.2	.7	.5	10	13
L.B.	1.8	1.2	.6	12	19
	1.7	1.0	.7	13	22
	1.8	1.3	.5	13	20
	1.8	1.2	.6	13	36
	2.1*	1.4	.7	19	19
R.F.	1.0	.6	.3	8	15
	.9	.5	.4	9	15
	.9	.5	.4	9	23
	1.3	.8	.5	15	13
R.B.	2.2	1.0	.6	39	67
	2.0	1.5	.4	38	64
	1.8	1.4	.4	37	65
	1.9	1.5	.4	34	175
	2.1	1.7	.4	64	70
D.L.	1.6	1.1	.5	8	12
	1.7	1.2	.5	8	11
	1.6	1.1	.5	8	12
W.H.	1.8	1.0	.8	10	16
	1.8	.8	1.0	10	13
J.C.	.8†	.2	.5	23	22
	.3†	.1	.2	16	24
	.3†	.2	.1	26	32
	.3	.3	.0	26	22

*7 days between treatment.

†Received total of 3000 mg for 16 days previous to fever therapy.

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[10] Farmer, C., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**. 146.

[11] Abbasy, M. A., Harris, L. J., Ray, S. N., and Marrack, J. R. O., *Lancet*, 1935, **1**, 1399.

Patients received, during the period of observation, 500 to 700 mg of ascorbic acid daily.* The heat treatments were given at intervals of 1-3 days and in one case an interval of 8 days elapsed between 2 of the treatments. A temperature of 105-106 degrees was maintained from 2-5 hours, depending upon the tolerance of the patient, and the usual routine to prevent water and chloride loss was followed.

Blood samples were drawn before and after the heat therapy, and on the intervening days. Twenty-four urine collections were made throughout the period of observations.

Table I gives results on these patients. It will be noted that there was a great deal of variation in the beginning blood levels, but each treatment depressed the level. The urinary excretion level was lower than normal in all but 2 cases and showed a small but consistent decrease on the day of treatment.

Table II contrasts the urinary excretions of normal subjects and this heat-treated group following the ingestion of 500 mg of ascorbic acid. The blood levels in both groups show much the same variation, but the normals excrete large amounts of the test dose. The pathological cases show very small excretion, less than the usual normal daily excretion. The normals excrete 30-40% of the ingested dose while these pathological cases, except in one instance, excrete only 2-4%.

TABLE II.
Urinary Excretion of Ascorbic Acid Following Test Dose of 500 Mg.

Subject	Blood Level, mg%	Urinary Ascorbic Acid, mg	% of Test Dose Excreted
	Normal Subjects.		
1	1.0	214	40
2	1.6	150	30
3	1.5	87	17
4	1.6	160	32
5	.9	195	39
	Pathological Subjects		
Cardiac	1.2	19	3
Obesity	1.0	26	5
Tuberculosis	.6	18	3
Arthritis	.5	25	5
Cardiac	1.0	20	4
	Fever Therapy Cases.		
1	.8	13	2
2	2.1	19	3
3	1.3	15	3
4	2.1	70	14
5*	.3	26	3

*Test dose—700 mg.

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* We wish to thank Berta Beyer Mathews for her assistance during the artificial fever treatments.

Summary. Elevated body temperature decreased the level of ascorbic acid in the blood, and also the excretion in the urine. This decreased excretion in the urine as found in this study and as reported for infectious cases with fever would seem to be due to the increased temperature and not to the invading organism.

10332

Relation of the Intact Pituitary Gland to Artificially Induced Ovulation.

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Among the Amphibia the anterior lobe of the pituitary gland has been shown to stimulate ovulation,[1-5] or amplexus on the part of the male[6-11] and sperm release has also been demonstrated.[12, 13] Gonadectomy caused morphological changes within the pituitary and hypophysectomy caused gonad atrophy.

It has further been shown[14] that toads will ovulate in response to the pituitary hormone even after hypophysectomy, or the removal of various major organs such as the adrenals, thyroids, kidneys, liver, alimentary tract or parts of the brain. The anterior pituitary hormone seems to act directly on the gonad without the intermedia-tion of any other organ through either the circulatory or the sympathetic systems. At least 7 variables have been found in this pituitary-ovulation relation[15] and it has long been known that ovarian response is not an all-or-none phenomenon. It was the purpose of this investigation to learn the relation of the intact pituitary to hormonal-induced ovulation in the frog, *Rana pipiens.*

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* This work was supported by a Grant-in-Aid from the Penrose Fund of the American Philosophical Society.

[1] Houssay, B. A., Giusti, L., and Lascano-Gonzalez, J. M., *Rev. de la Soc. Argentina,* 1929, **5**, 397.

[2] Wolf, O. M., *Anat. Record,* 1929, **44**, 206; Proc. Soc. Exp. Biol. and Med., 1929, **26**, 692.

[3] Adams, A. E., *Proc. Second Int. Cong. Sex Research,* 1930, 190.

[4] Rugh, R., *Biol. Bull.,* 1934, **66**, 22.

[5] Shapiro, H. A., *Nature,* 1935, **135**, 510.

[6] Houssay, B. A., and Giusti, L., *Compt. rend. Soc. de Biol.,* 1930, **104**, 1030.

[7] Bardeen, H. W., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 846.

[8] Rugh, R., *Biol. Bull.,* 1935, **68**, 74; *J. Exp. Zool.,* 1935, **71**, 163.

[9] Shapiro, H. A., and Zwarenstein, H., *So. Africa Med. J.,* March, 1935.

[10] Shapiro, H. A., *J. Exp. Biol.,* 1936, **13**, 48.

[11] Osima, M., *Sc. Rep. Tohoku Imp. Univ.,* 1937, **12**, 195.

[12] Houssay, B. A., and Lascano-Gonzalez, J. M., *Compt. rend. Soc. de biol.,* 1929, **102**, 866; *Rev. de la Soc. Argentina,* 1929, **5**, 77.

[13] Rugh, R., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 417.

[14] Novelli, A., *Rev. de la Soc. Argentina,* 1932, **8**, 454.

Mature hibernating frogs just received from Vermont were used in the experiments reported here. The anterior pituitary gland was removed, with the frog under ether anesthesia, by approaching it through the roof of the mouth and the parasphenoidal bone. The accompanying diagram shows the roof of the frog's mouth, with oral skin deflected to expose the parasphenoidal and the exoccipital bones and the first vertebra. The anterior pituitary gland is shown as it might be seen, in its normal position, through the parasphenoidal bone. This gland is exposed by 2 incisions, made at about a 30° angle, with sharp point of the scalpel inserted just posterior to the position of the gland. The triangular piece of bone is deflected forward and the anterior lobe of the pituitary gland is exposed and can be sucked up into a narrow-mouthed pipette. In this way the anterior lobe of the pituitary gland can be easily removed without disturbing other parts of the gland, and usually this lobe remains in a single piece. There is a minimum of bleeding if only the anterior lobe is exposed and removed. Care must be exercised, however, to avoid damage to the vena orbitalis posterior or to either of the palatine branches from the carotid artery. These vessels cross the transverse arm of the parasphenoidal bone. Following extirpation of the gland, the triangular flap of bone is replaced and the oral skin is sewed together, with silk thread, to facilitate healing. The controls were operated upon in the same manner, and the pituitary was exposed, but no tissue was removed.

The experiment was divided into 4 graded steps, as follows:

a. The female frog's anterior pituitary was broken up while in position, by means of a sharp needle. No injection.

b. The anterior pituitary was removed from the head and immediately injected into the abdomen.

c. The anterior pituitary was removed and kept in a modification of amphibian Ringer's solution[16] in the refrigerator for a period of 12 hours while the frog recovered completely from anesthetic and operative shock. Then this single pituitary was injected into the abdomen of the frog from which it had been removed.

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[15] Rugh, R., *Physiol. Zool.*, 1937, **10**, 84.

[16] Holtfreter, J., *Arch. f. Ent. Mech. der Org.*, 1931, **124**, 404.

d. Procedure same as "c" but the dose of pituitary hormone was increased by using in addition glands excised from other females.

In all cases where anterior pituitary hormone was injected, the procedure was the same as that recently described.[17] The doses recorded are in terms of whole female glands and ovulation was estimated in terms of percentage of the volume of egg mass within the uterus as compared with the total volume of egg mass determined by adding the volume of egg mass remaining within the ovary. The vitelline membrane on the uterine egg partially balanced the ovarian tissue surrounding the ovarian egg, at least to within the

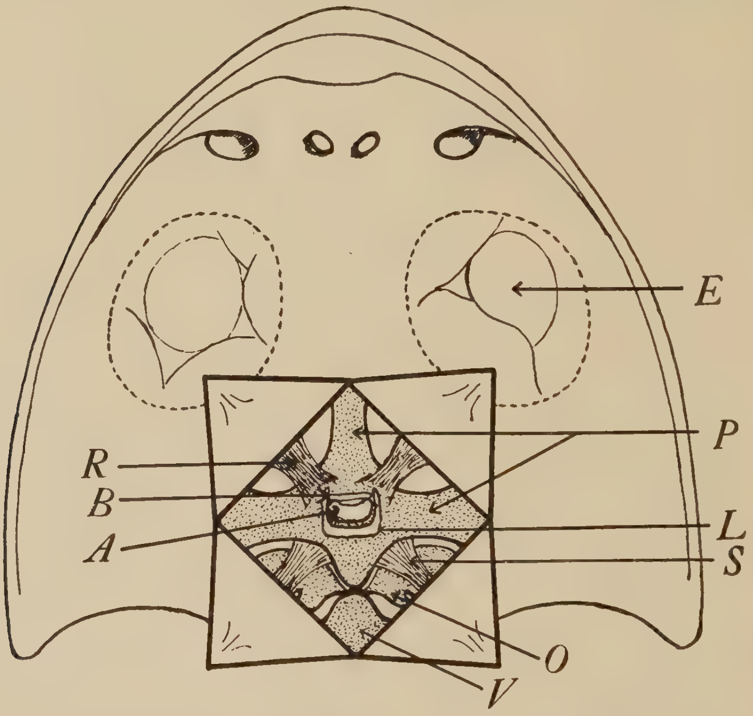

FIG. 1.

Diagram showing position of the pituitary gland of the frog as it might be seen through the parasphenoidal bone, lying just beneath the brain.

A—Anterior lobe of the pituitary gland. Note its more posterior position.
B—Pars intermedia and pars nervosa.
E—Eye ball as seen through oral skin.
L—Endolymphatic tissue adherent to the pituitary.
O—Exoccipital bone.
P—Parasphenoidal bone.
R—Retractor bulbi muscle. The attachment of this muscle to the parasphenoidal bone must be partially removed.
S—Levator anguli scapuli muscle.
V—First vertebra.

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[17] Rugh, R., *Science*, 1937, **85**.

limits of observational error, so that this minor factor was not considered. Data were secured 48 hours after hormone injection, a lapse of time found to be sufficient for the completion of ovulation as far as it will go, at laboratory temperatures of approximately 23-25°.

In 4 animals the anterior pituitary gland was broken up in position with a needle, without removal, after exposure as described above. Since this invariably entailed some bleeding it was thought that by this means the hormone might be made immediately available in the circulation. In none of these cases was any ovulation induced. It would hardly be justified, from these observations alone, to say that the host's pituitary would be inadequate to induce ovulation if liberated (as it must be in March) into the blood stream. This operative procedure gave negative results, although the frogs survived for many days.

In 7 animals the gland was removed and injected immediately (even before the frog recovered from the anesthesia) into the abdomen. The double operation (excision and injection) seemed to be too drastic for the frogs. Five died and 2 survived for 48 hours, but were definitely in very poor condition. No ovulation was achieved.

In 5 frogs the anterior pituitary gland was removed and placed in Holtfreter's solution[16] in the refrigerator for a period of 12 hours while the frog made a complete recovery from the anesthetic and the operation. Then this same gland was injected into the frog, inducing some ovulation in every case. The degree to which ovaries were emptied in different frogs varied somewhat. In one case almost complete ovulation was achieved, but the average corresponded well with the tabulated results below from another experimental series.

Since it was found that injection of the anterior pituitary hormone some time after hypophysectomy elicited ovulation, it was necessary to determine the quantitative difference, if any, between the anterior pituitary hormone and ovulation in normal and hypophysectomized frogs. Quantitative studies of this nature have been reported for

TABLE I.

Dose No. of female pituitaries	Controls—Avg % ovulation		Experimentals—Avg % ovulation	
1	0- 0	0	30- 80- 50- 50	52
2	20- 0	10	80- 80- 95- 90	86
3	30-50	40	100- 95-100-100	98
4	60-80	70	100-100- 95-100	98
8	100-95	98	95-100-100-100	98

Rana pipiens[15] but controls were run parallel with hypophysectomized frogs. The controls were operated upon but without gland removal, and received anterior pituitary doses comparable to the doses given to the experimental animals. The results are shown in Table I.

In a subsequent paper the effect of pituitary-induced ovulation on the host's pituitary will be described. Thus far it has been assumed that the host's anterior pituitary gland plays, if anything, a passive rôle in ovulation induced during the hibernation period. It has even been suggested that the hormonal content of the anterior pituitary gland of the hibernating frog may be deficient in respect to the sex stimulating factor. This can hardly be the case since glands from hibernating frogs can be used to induce ovulation as early as the latter part of August in *Rana pipiens.* Also, as indicated above, the host's gland alone is sufficiently potent to induce 50% or more ovulation during hibernation. Recently, hibernating males have been seen to attempt amplexus shortly after being transferred from hibernation temperatures to a level of 11°-14°C. The frog's gland is adequate to cause complete ovulation in March, and it is quite possible that with greater care in the removal, storage, and injection of the one gland that complete ovulation may be achieved during hibernation. It must be admitted, however, that as breeding approaches there may be significant physiological changes both within the pituitary and within the frog as a whole.[15, 18]

While this pituitary-gonad relation requires further investigation, there appear 2 possible interpretations of the above data. First, the recipient's intact pituitary gland may in some way inhibit the action of added hormone material and this inhibition may be experimentally overcome only by an increase of 300-400% in dosage. Second, there is the hypothetical conception of an inter-relationship between the anterior pituitary gland and the gonads which, when interrupted by the extirpation of one part of the circuit, allows freer invasion of that circuit.

Conclusion. The normal hibernating frog may be induced to ovulate 50% or more of its eggs by the injection of 3 to 4 anterior pituitary glands from female frogs, but if the frog is first hypophysectomized and then allowed to recover completely from the operation, its own gland (or one from another female) will elicit the same degree of ovulation.

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[18] Zahl, P., *Z. f. Mikrosk. Anat. Forsch.*, 1937, **42**, 303.

10333 P

Colchicine Reactions in Ventral Prostate of Castrated Male Rats Following Androgenic Treatment.*

ELIZABETH Z. BURKHART. (Introduced by C. R. Moore.)
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Since Lits[1] and Dustin[2] showed that colchicine arrests cell division, several workers have utilized the drug to study phases of reproductive processes and hormone activity in the female mouse,[3-6] rabbit,[7] pigeon,[8] and the male rat,[9, 10] and guinea pig.[11, 12]

I have employed colchicine to study effects of male hormone on the ventral prostate of castrated male rats. The dosage was fixed at 0.1 mg per 100 g weight of rat (0.1 cc of a 1 mg/cc aqueous solution). Testosterone propionate was injected into 80-day-old rats castrated on day 40.

Experiment I. The castrated members of 2 litters were given 3 daily injections of 0.1 mg of testosterone propionate; on the 4th day each animal was injected with colchicine. Castrated controls treated with colchicine were available. At 2-hour intervals following subcutaneous injection of colchicine one animal from each litter was sacrificed. Coagulating glands, seminal vesicles and ventral prostates were removed. Over 1000 nuclei in the glandular epithelium of each ventral prostate were counted (Table I).

During the first 4 hours after colchicine injection all mitotically active cells showed the effect of colchicine by scattering or clumping the chromosomes without spindle formation as described in nor-

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* This investigation has been aided by a grant from the Rockefeller Foundation to the University of Chicago. Testosterone propionate was provided by Dr. Gregory Stagnell (Schering Corporation).

[1] Lits, F., *Compt. rend. Soc. de biol.*, 1934, **115**, 1421.

[2] Dustin, A. P., *Bull. Acad. roy. de méd. de Belgique*, 1934, **14**, 487.

[3] Allen, E., and Creadick, R. N., *Anat. Rec.*, 1937, **69**, 191.

[4] Allen, E., Smith, G. M., and Gardner, W. U., *Am. J. of Anat.*, 1937, **61**, 321.

[5] Rogers, P. V., and Allen, E., *Endocrinology*, 1937, **21**, 629.

[6] Lits, F., *Compt. rend. Soc. de biol.*, 1935, **118**, 393.

[7] Allen, E., Smith, G. M., and Reynolds, S. R. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 257.

[8] LeBlond, C. P., and Allen, E., *Endocrinology*, 1937, **21**, 455.

[9] Bastenie, P., and Zylberszac, S., *Compt. rend. Soc. de biol.*, 1937, **126**, 891.

[10] Bastenie, P., and Zylberszac, S., *Compt. rend. Soc. de biol.*, 1937, **126**, 1283.

[11] Bastenie, P., and Zylberszac, S., *Compt. rend. Soc. de biol.*, 1937, **126**, 1282.

[12] Jailer, J. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 281.

TABLE I.

Litter No.	Hr after injection	Resting nuclei	Clumped colchicine figures	Scattered colchicine figures	Prophase nuclei	Clusters of small nuclei	Total	Percentage of colchicine figures
1	2	1250	12	51	20	0	1333	4.72
	4	1641	28	82	7	0	1758	6.25
	6	1072	88	43	17	2	1222	10.71
	8	1094	105	11	11	10	1231	9.41
	10	1352	145	3	29	11	1540	9.60
	12	1587	78	0	31	16	1712	4.55
2	2	1006	12	37	7	0	1062	4.61
	4	1185	56	134	4	0	1379	13.05
	6	1165	137	49	4	0	1355	13.50
	8	889	122	19	10	24	1064	13.25
	10	956	85	2	6	7	1056	8.23
	12	1034	39	1	19	9	1102	3.62

mal and tumorous mouse tissue[13] and in plants.[14] Prophase stages appeared unaffected 12 hours after injection. By the 6th hour most colchicine figures were of the clumped type and the total number of mitotically active cells reached its height. This mitotic index peak appears 3½ hours earlier than in transplanted mouse tumors.[2, 15] At 8 hours and thereafter abnormal clusters of small nuclei were present and the total number of colchicine figures gradually decreased.

Six hours was selected as the optimal period of colchicine treatment for study of the ventral prostate in the following experiment:

Experiment II. Of 29 males castrated on day 40, 11 received 0.1 mg testosterone propionate on day 80 and were killed at 4-hour intervals in pairs; one of the pair received colchicine 6 hours prior to autopsy, whereas the second animal of the pair received only androgen (Series I, Table II). Sixteen of the castrate males received a second injection of testosterone propionate on day 81 (Series II) and otherwise were handled as those of Series I. Two animals served as castrate controls. Mitotic figure counts were made under the 4 mm lens and an ocular micrometer disc with a field divided into 49 squares. The results are recorded as averages of the mitotically active cells per field.

Mitotic figures are not significantly increased until 36 hours after androgen injection. Only one mitotic cell was found in 50 fields in

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[13] Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **18**, 411.

[14] Eigsti, O. J., *Proc. Nat. Acad. Sc.*, 1938, **24**, 56.

[15] Dustin, A. P., Leeuwenhoek-Vereeniging, IVieme Conference tenue à Amsterdam, Juni, 1935.

TABLE II.

	Average No. of mitotic figures per field (50 fields)			
	Series I 0.1 mg test. prop.		Series II 0.2 mg test. prop.	
Time of autopsy after 1st dose of test. prop.,hr	Control	Colchicine treated	Control	Colchicine treated
15	—	.02		
19	.00	.02		
22	.02	.04		
27	.02	.10		
32	.04	.70	.06	.406
36	1.04	.40	1.50	.504
40			3.08	26.64
44			2.82	32.72
48			2.00	30.62
52			.66	20.88
55			2.54	18.74
60			3.44	40.18

colchicine-treated animals killed at 15 and 19 hours. One can find, very rarely, a mitotic cell in an untreated castrate control rat of the same age. Mitotic activity is highest between 40 and 44 hours, falls, and increases again at 60 hours. Work now in progress will show whether the second increase is due to the second dose of androgen.

Thirty-six hours after the first dose of testosterone propionate there is a sudden increase in the number of mitotic cells in the non-colchicine-treated animals while a corresponding increase after colchicine treatment occurs at 40 hours. This characteristic lag in activity in the colchicine-treated gland suggests that colchicine, having arrested mitosis during the early part of treatment, interferes with the normal mitotic rate just before the animal is sacrificed. Further work is necessary to determine the significance of this interference.

Conclusion. Mitotic activity in rat ventral prostate gland cells is studied to advantage 6 hours after colchicine administration. Testosterone propionate (0.1 mg daily) initiates cell division in castrate prostates 36 hours after injection; activity is maximal at 44 hours, subsides, and rises again. The action of colchicine is apparent only in dividing cells.

10334

Effect of Temperature on Activity of Choline-Esterase.*

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Kahane and Lévy[1] observed a temperature optimum for the combined enzymatic and non-enzymatic hydrolysis of acetylcholine in the presence of horse serum extending from 38° to 60°. In the present investigation the purely enzymatic activity was found to have a very narrow optimum at 40°. Apparently the broad maximum found by Kahane and Lévy was the resultant of increased non-enzymatic hydrolysis occurring as the temperature was elevated, and coexistant decreased enzymatic scission. Early studies on the heat-inactivation of choline-esterase were carried out by Abderhalden and Paffrath,[2] who found that the choline-esterase in the press-juice of the small intestine of the pig was unaffected by heating at 55-58° for 5 minutes, whereas after 2 hours a certain loss of activity resulted. Heating for 5 minutes at 70-75° caused total destruction of the enzyme. Loewi and Navratil[3] found that frog heart extracts had a less destructive action upon acetylcholine when they were heated at 56° for 20 minutes. Plattner and Hintner[4] later confirmed the gradual inactivation of the enzyme in organ extracts when the latter were held at 56°.

Balls and Matlack[5] observed that the splitting of benzyl butyrate or stearate by pancreas extracts changes from a zero order reaction at 40° to more of a monomolecular reaction as the temperature is lowered. Experiments presented in this paper show that the course of the enzymatic scission of acetylcholine is unaffected by a temperature change from 40° to 25°.

Because of the ease with which the course of the hydrolysis could be followed, the manometric method employing the Warburg apparatus was used for these experiments in a manner essentially the same as that previously employed.[6] Correction for non-enzymatic hydrolysis was made in every case. One-half ml of 2% horse serum was used in the side arm of the reaction flask and 1.5 ml of acetyl-

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* This work was aided by a grant from the William Antopol Research Fund.

[1] Kahane, E., and Lévy, J., *Compt. rend. Acad.*, 1936, **202**, 781.

[2] Abderhalden, E., and Paffrath, H., *Fermentforsch.*, 1925, **8**, 299.

[3] Loewi, O., and Navratil, E., *Arch. ges. Physiol.*, 1926, **214**, 678.

[4] Plattner, F., and Hinter, H., *Arch. ges. Physiol.*, 1930, **225**, 19.

[5] Balls, A. K., and Matlack, M. B., *J. Biol. Chem.*, 1938, **125**, 539.

[6] Glick, D., *J. Biol. Chem.*, 1938, **125**, 729.

choline chloride solution (5 mg per ml) in the main chamber. Readings were taken every 10 minutes for 60 minutes to obtain the slope of the initial linear reaction curve, and the points in Fig. 1 represent the hydrolyses after 60 minutes. The course of the reaction was linear for the 60-minutes period at all temperatures except 50°. In this case an extrapolation from the linear portion to the 60-minutes point was made. The temperature coefficients in Table I were calculated from data taken from Fig. 1. In order to observe the effect upon the course of the reaction of a change from 25° to 40°, an experiment was performed using only 3 mg of substrate in each vessel. The quantity of substrate was reduced in order that a shorter time be required for following the reaction to about 50% of the total hydrolysis. The temperature constants in Table II were calculated from these data.

The curve for the effect of temperature upon the activity of choline-esterase (Fig. 1) drops sharply on both sides of the optimum. The temperature coefficient, which is the ratio of the enzymatic activity at a given temperature to that at a temperature 10° lower. may be seen to fall to a small extent as the temperature increases toward the optimum; this behavior is common for hydrolytic enzymes. Clark, Raventós, Stedman and Stedman[7] found the rate of

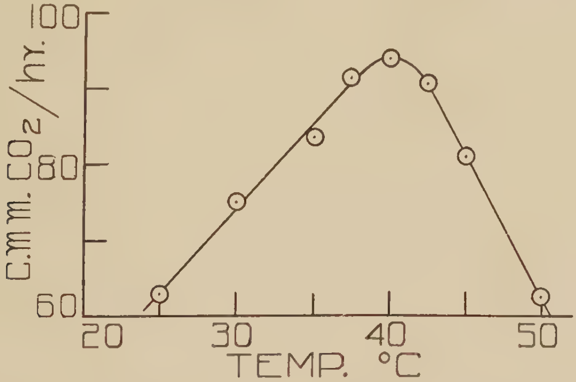

FIG. 1.
Effect of Temperature upon the Activity of Choline Esterase.

TABLE I.
Temperature Coefficients of Choline Esterase.

Temp. range, C°	$K_{t+10} : K_t$
25-35	1.36
30-40	1.27
40-50	0.66

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[7] Clark, A. J., Raventós, J., Stedman, E., and Stedman, E., *Quart. J. Exp. Physiol.*, 1938, **28**, 77.

TABLE II.
Velocity and Temperature Constants of Choline Esterase.

t min	25° mm3 CO_2 evolved	25° k_1	40° mm3 CO_2 evolved	40° k_2	Calories*
60	55	.00268	80	.00405	5100
120	103	.00272	144	.00411	5100
180	145	.00276	194	.00412	4950

$*A = \frac{\log k_2 - \log k_1}{0.4343} R \frac{T_2T_1}{T_2 - T_1}$. k_1 and k_2 are velocity constants, R is the gas constant, and T_1 and T_2 are absolute temperatures referring to k_1 and k_2 respectively.

hydrolysis at 18° to be two-thirds that at 37°. The magnitude of the coefficients is of the same order as that found by Kastle and Loevenhart[8] for the action of pancreas and liver extracts upon ethyl butyrate, namely 1.34 (10-20°), 1.26 (20-30°), and 1.36 (10-20°), 1.10 (20-30°) respectively. Terroine[9] obtained higher values for the hydrolysis of ethyl butyrate by pancreas: 2.03 (5-15°), 1.96 (15-25°).

It was observed that the rate of enzymatic hydrolysis at 50° fell off rapidly after 45 minutes, whereas at 45° the reaction was still linear after 90 minutes. A progressive destruction of enzyme by heat was no doubt the basis of this effect. There seem to be two factors operating: a lowered activity independent of heat destruction and requiring no time interval to become manifest, shown by the decrease in the slopes of the linear portion of the reaction curves at 42.5 and 45° as compared to that at 40°, and a gradual destruction of enzyme by heat, demonstrated in addition to the first effect, at 50°.

The temperature change from 25° to 40° had little effect upon the course of the hydrolysis. During the second hour the reaction deviated from the linear to an essentially monomolecular one. Velocity constants calculated for the second and third hours according to the monomolecular formula: $k = 2.3/t \log a/a\text{-}x$ were in turn used to calculate temperature constants. (Table II). The values found fell between those reported for ester hydrolysis by Kastle, 4100, and Terroine, 11,600, quoted by Haldane.[10]

Summary. The activity-temperature relationship for choline-esterase in horse serum was investigated and a narrow optimum at

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[8] Kastle, J. H., and Loevenhart, A. S., *Am. Chem. J.*, 1900, **24**, 491.

[9] Terroine, E. F., *Biochem. Z.*, 1910, **23**, 404, 429.

[10] Haldane, J. B. S., *Enzymes*, New York and London, 1930, 67.

40° was observed. The decreased activity above 40° is believed to result from two factors, a slowing of the reaction independent of enzyme destruction, and destruction itself, first found to occur at 50°. The course of the reaction was not appreciably affected by a change in temperature from 25 to 40°. Temperature coefficients and constants for the action of this enzyme were calculated.

The author wishes to thank Mr. Sidney Morett for technical aid in this investigation.

10335

Germicidal Action of Bromine.

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The halogens, fluorine, chlorine, bromine, and iodine are strong oxidizing agents which combine readily with protoplasm. Chlorine is widely used as a disinfectant in water-treatment while iodine is a much used skin-disinfectant. Fluorine is probably too active to use in disinfection. Less work has been done with bromine, which strongly resembles chlorine in its general properties. Review of the literature gives little information on relative resistance of pure cultures of microörganisms to bromine under known conditions. Beckwith and Moser[1] reported that chlorine, bromine, and iodine were about equally effective toward *Escherichia coli,* that is, 0.2 parts per million of each halogen showed 95 to 97% efficiency in 60 minutes. Wood and Illing[2] found that after 5 minutes, water containing *Escherichia coli* was sterile in presence of 0.15, 0.35, and 0.45 ppm. Henderson[3] patented a device for "antisepticizing" water with bromine. Hildesheim[4] used bromine for treatment of swimming-pool water. Bromine has also been used as a seed-disinfectant by DeZeeuw,[5] Wilson,[6] Matskov,[7] and LaRue.[8] Other work has been carried out by Regel,[9] Shuhmacher,[10] Friedenthal,[11] Rupp, Wegner, and Maiss.[12]

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[1] Beckwith, T. D., and Moser, F. R., *J. Am. Water Works Assn.,* 1933, **25**, 267.

[2] Wood, R. D., and Illing, E. T., *Analyst,* 1930, **55**, 126.

[3] Henderson, C. T., U. S. Patent, 1935, 1,995,639.

[4] Hildesheim, H., *Tech. Gemeindeblatt,* 1936, **39**, 56.

[5] DeZeeuw, R., *Exp. Sta. Rec.,* 1912, **26**, 819.

[6] Wilson, J. K., *Am. J. Bot.,* 1915, **2**, 420.

[7] Matskov, F., Physiol. Untersuch. Zuckerrübe Erste Artikelserie, Ukrainisches Inst. Angew. Bot. Sect. Pflanzenphysiol. Charkiv 156-190, 1930.

[8] La Rue, C. D., *Science,* 1937, **85**.

The organisms used in this investigation are indicated in the tables. The germicidal value of bromine is expressed in ppm required to kill a suspension of the organisms under controlled experimental conditions. The smallest amount of bromine required to kill each organism within a certain time is the minimal bromine death-point. A stock saturated solution of bromine was prepared

TABLE I.

Amount of Free Bromine Required to Kill Spores of Aërobic Spore-forming Bacteria in 30 Seconds.

Organism	Parts per million of Bromine	
	pH 3.5-4.0	pH 6.8-7.2
Proteus vulgaris	40-60	170
Bacillus megatherium	28-35	110
” *mesentericus*	180-300	over 450
” *subtilis* (565)	160-200	over 450
” ” (566)	170-220	over 450

TABLE II.

Amount of Free Bromine Required to Kill Mold-spores, Yeasts, Non-spore-forming Bacteria in 15 to 30 Seconds, at Room Temperature.

Organism	Bromine ppm
Kill mold-spores:	
Aspergillus niger	28
” *sp.*	25
Oöspora lactis	8
Mucor sp.	30
Penicillium sp.	1-5
Yeasts:	
Cryptococcus	.3
Mycoderma	.5
Endomyces albicans	*
Monilia albicans	.25
Saccharomyces sp.	3.0
Non-spore-forming bacteria:	
Staphylococcus aureus (92)	.15
” *albus* (76)	.25
” *sp.* (80)	.20
” *aureus* (77)	.15
” ” (79)	.10
” ” (89)	.15
Escherichia coli (252)	.15
” ” (251)	.15
Eberthella typhosa (377)	>.03
” ” (378)	.06

*Results inconsistent.

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[9] Regel, *Deut. Militärarztl. Z.*, 1914, **43**, 55; *Chem. Zentr.*, 1914, **1**, 581.

[10] Shuhmacher, *Schweig. Apoth. Ztg.*, 1915, **53**, 281.

[11] Friedenthal, H., Brit. Patent 215,016, 1923.

[12] Rupp, E., Wegner, W., and Maiss, P., *Arch. Pharm.*, 1924, **262**, 2.

before each experiment. A suspension of the microörganism under study was treated with the bromine solutions in 50 cc quantities and counts of viable organisms made at regular intervals. A suspension in water was counted as a control at the same time. One cc of the inoculated bromine water was plated at intervals of 30 seconds, 1, 2, 3, and 5 minutes. A small amount of sodium-thiosulfate solution was previously added to the dish to stop the action of the bromine.

Acidity was found to be important. Bromine was more active at a pH 3.5-4.0. When the reaction was changed to neutrality much more bromine was required to kill the organisms. The results with aërobic spore-bearing bacteria are shown in Table I.

Aërobic spore-forming bacteria are almost as resistant to bromine as they are to chlorine. They are also much more resistant to bromine than are non-spore-forming bacteria, mold-spores and yeasts.

Mold-spores are quite resistant to bromine. They survived exposure to concentrations that readily killed yeasts and non-spore-forming bacteria.

Conclusions. Bromine is shown to be an effective germicidal agent against non-spore-forming microörganisms. Spore-forming bacteria are much more resistant. The element has marked specificity for *Escherichia coli* and *Eberthella typhosa*. Mold-spores were more resistant than yeasts or non-spore-forming bacteria.